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Research Article

Biomonitoring of Heavy Metals in the Guava (*Psidium guajava*) for Their Health Risk Assessment in Kluang, Malaysia

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Abstract: This study aimed to investigate the concentrations of copper (Cu), zinc (Zn), nickel (Ni), lead (Pb) and iron (Fe) in different parts of guava (pulps, peels and seeds) and its topsoil collected from Kluang, Malaysia, and to assess their effects on human health. The level of metals was determined using acid digestion method and atomic absorption spectrometry. The results showed that the concentrations of Cu, Zn and Pb in all parts of guava was above the World Health Organization (WHO) permissible limits. However, the value of heavy metals in the topsoil were below the WHO limit. The concentration order of the metals in the guava's pulps was Fe > Cu > Zn > Pb > Ni. The contamination factor (*Cf*) calculated from the topsoil was low for Cu, Zn and Ni and moderate for Pb. However, potential ecological risk (*Er*) of individual metal ranged from 0.25-7.58 for Cu, Zn, Pb and Ni was low. The potential non-carcinogenic health risk for consumers was assessed using estimated daily intake (EDI) and target hazard quotient (THQ). The contribution of heavy metals to the EDI for all consumers followed the order of Fe > Cu > Zn > Pb > Ni. The THQ values were all below 1 for all five metals tested, indicating no potential risk to the consumers. We suggest that the Kluang's guava is safe to consume. However, regular monitoring and human health risk assessment of heavy metals in the guava is recommended to be carried out.

Keywords: Guava, heavy metals, ecological risk, health risk, carcinogenic

1. Introduction

Fruits are important for human health and nutrition, mainly as source of vitamins. Guava (*Psidium guajava*) is well known tropic tree which is abundantly grown for fruit [1]. The tree originated from southern Mexico through Central America. It has a wide ecological adaptation toward different soil types and climates, and therefore its fruits are abundantly available in many tropical and sub-tropical regions of the world [2]. The fruits contain high medicinal values and are rich in vitamin A, C, iron, phosphorus, calcium and minerals [1, 3]. Guava can also be categorised as medicinal plant, since it has been used in many countries for the treatment of diarrhoea, dysentery, gastroenteritis, hypertension,

diabetes, caries and pain relief [1]. In Malaysia, the subject fruit is regularly consumed by the public and can be easily found at local markets and fruit stalls.

A general pathway of heavy metal exposure to human is through daily intake of food crops, including fruits and vegetables. The consumption of food contaminated with heavy metals could threaten the human health. Continuous consumption of food that are contaminated with high level of heavy metals can pose health risk to normal body function and cause many diseases such as cardiovascular, nervous, kidney, liver, and bone diseases in human [4]. Also, prolonged consumption of food with low level of heavy metals has a detrimental influence on human health, and within years of accumulation, the negative effect becomes obvious [5]. Hence, heavy metal contamination has become a major concern across the world.

Monitoring of heavy metal levels in fruits and vegetables is vital for health risk assessment during food consumption. Many countries have conducted regular monitoring to establish information on heavy metals in food. Numerous papers have documented the metals concentration in the fruits [6-9] and vegetables [4, 10-20], yet only a few in edible guavas [7, 21-24]. Thus, it is essential to evaluate the chemical composition of this guava and make known of their impact on public health.

The objective of this study is to determine the concentrations of some selected heavy metals i.e. copper (Cu), zinc (Zn), nickel (Ni), lead (Pb) and iron (Fe) in the pulps, peels and seeds of guava as well as their habitat topsoil collected from Kluang, Malaysia. The health risk associated with these heavy metals to consumers were also evaluated.

2. Materials and methods

The sampling for guava was conducted on 1 December 2015, at Taman Kekal Pengeluaran Makanan (Permanent Food Production Areas), located in Kluang, Johor, on the southern part of Peninsular Malaysia (1°55′59.2428″ N; 103°11′8.8152″ E). Ripe and unripe guavas were randomly collected from five individual trees (Table 1). The fruits were sorted, labelled and stored in polystyrene containers. The habitat topsoil samples were collected, placed in sealable polyethylene plastic bags and put in an iced box. All samples were transported to the laboratory in Universiti Putra Malaysia (UPM), Serdang, Selangor for acid digestion.

Table 1. Description on measurement of guava (*Psidium guajava*) and its habitat topsoil collected from of guava plantation in Kluang

Tree	Tree Height (m)	Fruit Diameter (cm)	Fruit Weight (g)	Topsoil depth (cm)
B1	4-5	18.0	100	10.0
B2	4-5	30.7	450	10.0
B3	4-5	31.4	510	10.0
B4	4-5	28.3	370	10.0
B5	4-5	28.3	380	10.0

At the laboratory, the fruits were washed, cleaned, cut and separated into fleshy pulps, peels (skins) and seeds, following the method by [22]. The separated fruit parts and topsoil were oven dried to constant weight for 72 hours at 60°C. Then, the dried samples were crushed to powder using a mortar and pestle. The powdered samples were sieved through a 63 μm stainless steel sieve.

Approximately 1 g of different dried parts (peels, pulps and seeds) were placed in separate digestion tubes and dissolved in 10 mL of concentrated nitric acid (HNO_3; AnalaR grade; BDH 69%). For topsoil, 1 g of its dried samples were digested in a combination of concentrated nitric acid (HNO_3; AnalaR grade; BDH 69%) and perchloric acid ($HClO_4$; AnalaR grade; BDH 60%) in the ratio of 4:1. The tubes were heated for 1 hour on a hot block digester at 40°C before the temperature was raised to 140°C, where the contents were fully digested after 3 hours. The digested samples were then diluted up to 40 mL with distilled water and filtered with Whatman's No.1 filter papers. The filtered solutions were kept in acid washed pill boxes. All experiments were carried out in triplicates, using the method highlighted by [25].

The samples were digested in aqua regia and the concentrations of Cu, Zn, Ni, Pb and Fe were determined using an air acetylene flame atomic absorption spectrophotometer (AAS) model Thermo Fisher Scientific Spectrometer (S Series GE712405 v1.27). The recoveries of certified reference materials for MESS-3 were Cu (101%), Zn (57.7%), Ni (78.9%), Pb (107%) and Fe (53.9%). The results were presented in mg/kg dry weight of sample, following Yap et al. [25].

2.1 *Ecological risk assessment*

The contamination factor (Cf) was calculated for each pollutant. Cf requires at least five surficial sediment samples are averaged to produce a mean pollutant concentration which then compared to a baseline pristine reference level [26-28], as follows:

$$Cf = Cd / Cr$$

Where Cd is the concentration of the metal for this study, Cr is the reference concentrations (mg/kg dry weight) based on continental crust reference values (Cu = 25; Zn = 65; Ni = 56; Pb = 14.8) [29].

The potential ecological risk (Er) of individual metals, except for Fe, was estimated using the formula proposed by [26], as follows:

$$Er = TRF \times Cf$$

The toxic-response factor (TRF) used were Cu = 5, Zn = 1, Ni = 5 and Pb = 5. However, the Er for Fe was not calculated because the TFR was not established [26].

Lastly, the potential risk of individual (PERI) metal was calculated as the summation of all Er values [26, 28].

2.2 *Health risk assessment*

The dry weight basis concentration of the heavy metal obtained previously were converted to wet weight basis using a conversion factor of ratio dry weight to wet weight of sample. The conversions to wet weight basis are based on conversion factors of 0.12 and 0.19 for ripe pulps and unripe pulps, respectively. The dry weight concentration for each metal was then multiplied with conversion factor to get the wet weight basis concentration. In order to assess the health risks through consumption of guava, formulas highlighted by [13] and [25] were used. The estimated daily intake (EDI) was calculated based on the equation below:

$$EDI \ (\mu g/kg/day) = (Mc \times CR) / ABW$$

Where Mc is the metal concentration in wet weight basis (mg/kg), CR is the consumption rate for average-level consumer (ALC) and high-level consumer (HLC) (g/day), and ABW is the average body weight (60 kg for adult; [25]) (19.25 kg for children; [11]).

Yen ST, et al. [30] reported that the consumption rate of fruits for average-level consumer (ALC) and high-level consumer (HLC) were 150 and 300 g/day respectively for adults. While for children, the consumption rate for ALC and HLC were lower at 50 and 100 g/day respectively.

Estimation of non-carcinogenic risk of heavy metals consumption was determined using target hazard quotient (THQ). THQ is defined as the ratio between EDI and ORD, as follows:

$$THQ = EDI / ORD$$

Where ORD is the oral reference dose ($\mu g/kg/day$).

The values of ORD ($\mu g/kg/day$) used in this study for Cu, Zn, Ni, and Fe were 40, 300, 20, and 700 respectively [25, 31]. The ORD for Pb was based on provisional tolerable daily intake as 3.57 [32]. A THQ lower than 1 indicates an acceptable level of non-carcinogenic effects on health, whilst THQ of 1 or more indicates an unacceptable level of risk [25].

3. Results and discussion
3.1 *Metals in the guava*

The mean data of heavy metal concentrations in the pulps, peels and seeds of guava and its habitat topsoils are presented in Table 2. The level of heavy metals varied between different parts of guava and between different conditions (ripe and unripe). The metal concentrations (mg/kg dry weight) in the edible ripe pulps were Cu (23.8), Zn (9.39), Ni (2.44), Pb (3.91) and Fe (40.9) while in the unripe pulps were Cu (4.52), Zn (8.15), Ni (2.03), Pb (3.91) and Fe (38.6). Overall, the values of Cu, Zn, Ni and Fe ripe pulps were higher than those in the unripe pulps. For peels, the values of Cu and Zn in the ripe peels were higher than those in the unripe peels. For seeds, the values of Cu and Pb in the ripe seeds were higher than those in the unripe seeds while Zn and Fe levels in the unripe seeds were higher than those in the ripe seeds.

Table 2. Heavy metal concentrations (mg/kg dry weight except for those indicated *)
in the pulps, peels and seeds of guava (*Psidium guajava*) and its habitat topsoil collected from of guava plantation in Kluang. N = 3

Parts		Cu	Zn	Ni	Pb	Fe
Pulp	Ripe	23.8 ± 3.87	9.39 ± 0.76	2.44 ± 0.55	3.91 ± 0.73	40.9 ± 1.27
	Unripe	4.52 ± 0.53	8.15 ± 0.12	2.03 ± 0.41	3.91 ± 0.98	38.6 ± 0.95
Pulp*	Ripe	2.86	1.13	0.29	0.47	4.91
	Unripe	0.86	1.55	0.38	0.74	7.33
Peel	Ripe	58.2 ± 12.5	19.0 ± 5.48	2.81 ± 1.05	2.73 ± 2.48	45.9 ± 4.44
	Unripe	37.6 ± 20.3	11.4 ± 1.24	2.46 ± 0.01	2.97 ± 0.01	41.7 ± 0.69
Seed	Ripe	28.0 ± 6.56	14.5 ± 1.61	0.79 ± 0.45	9.09 ± 3.42	27.4 ± 1.23
	Unripe	18.3 ± 2.15	19.0 ± 0.52	0.89 ± 0.44	3.59 ± 0.01	36.9 ± 6.13
Topsoil		10.6 ± 0.33	32.6 ± 0.57	2.54 ± 0.35	23.16 ± 4.58	35458 ± 1957
WHO permissible limit in plant		10	0.60	10	2	
WHO permissible limit in soil		36	50	35	85	

Note: * = wet weight basis; values are expressed as mean ± standard deviation; numbers in bold represent values above WHO limit.

In general, the concentrations of Cu, Zn and Pb in all parts of dried guava were above the World Health Organization (WHO) permissible limits of 10, 0.60 and 2 mg/kg respectively. However, this study also revealed that Cu, Zn, Ni, and Pb concentration in the topsoil of Kluang were all below the WHO permissible limits of 36, 50, 35, and 85 mg/kg respectively.

In another local guava plantation situated in Bidor, Perak, on the northern part of Peninsular Malaysia, Ang and Ng [21] reported that the metal concentrations (mg/kg dry weight) in the seedless guava as Cu (4.61 for agricultural land; 3.74 for ex-mining land), Zn (11.6; 8.74), Ni (0.18; 0.55), and Pb (2.44; 8.71). Outside Malaysia, Waheed et al. [7] reported that the concentrations of Fe and Zn in the guava from local markets of Pakistan were 70 mg/kg and 29.5 mg/kg dry weight respectively. Yami et al. [23] reported that the metal concentrations (mg/kg dry weight) in the edible portion of guava collected from Nura Era Farm of Ethiopia as Cu (1.20), Zn (1.40), and Fe (5.60). Another study in Pakistan by Abrar et al. [24] discovered that the values of Pb in guava planted along Sagian Wala Bypass, Lahore, were ranged from 6.57 to 13.10 mg/kg. Thus, it is sensible to assume that guava is a good heavy metal accumulator.

The results showed that different parts of guava accumulate these metals at different concentrations. The concentrations of Cu, Zn, Ni and Fe in the peels were higher than those in the seeds and pulps (except for unripe seeds). However, for Pb, the value was higher in seeds than those in peels and pulps. This could be explained by the different ability of metal accumulation in the different tissues, pesticides residues, and atmospheric deposition on the surface of the fruit. The higher metal levels in the peels suggest that total washing and peeling off the fruits are needed before consuming the fruits. Nonetheless, the higher level of Pb in the pulps than the peels indicates the needs for regular monitoring of heavy metals for consumer safety.

The levels of Cu, Zn, Ni and Fe were higher in the ripe pulps and peels than in the unripe pulps and peels. In seeds, the levels of Cu and Pb were greater in the ripe seeds than those in the unripe seeds, while levels of Zn, Ni and Fe were higher in the unripe seeds than those in the ripe seeds. The different levels of metals between ripe and unripe parts of guava could be due to differences in water content and the maturity stage of the tree that bear the fruits [9]. Yang et al. [10] stated that the factors influencing the metal levels in plants and habitat topsoil which included soil condition, climate, irrigation, agricultural practices, atmospheric deposition, degree of maturity of the plant and fruit at the time of harvesting. The relatively lower levels of Cu, Zn, Ni, and Fe in the guava fruits (both ripe and unripe) could be attributed to these nutrients are required for plant growth with good management practices. The application of pesticides and fertilizers in this plantation followed the recommended dosages.

The abundance of heavy metals in the ripe guava's pulps was in the order: Fe > Cu > Zn > Pb > Ni. The metals were dominated by Fe, Cu, and Zn as these nutrients play major roles in optimal plant growth and major enzymatic activities [33]. Zn acts as an important activator of enzymes in synthesizing the hormone precursor [34], while Fe is essential in chlorophyll synthesis and photosynthesis [33].

Fe content in guava was found abundant in peels followed by pulps and seeds. The trend was differed than the previous study by [22], where Fe was found abundant in seeds followed by peels and pulps. Pulps of guava obtained from agricultural land in Bidor showed distribution of Zn, Cu and Pb of 11.62, 4.61 and 2.44 mg/kg dry weight respectively [21]. According to Radwan and Salama [8], the concentration of Zn and Cu were the highest compared to Pb and Cd in multiple fruits species sampled from a local market in Alexandria city, Egypt.

Fe was the most abundant metal in the topsoil followed by Zn, Pb, Cu and Ni with the values of 35458, 32.6, 23.2, 10.6, and 2.54 mg/kg respectively. The excessive level of Fe could be due to its present in the earth crust. According to [27], Fe has high natural concentration in soil and does not enrich from anthropogenic sources. The low Cu levels in Kluang's topsoil could be due to the acidic type of soils caused by the leaching of Cu at the surface (0-30cm), which then increases the acidity in the soil [35].

3.2 Ecological risk assessment

The values of Cf, Er, and PERI are presented in Table 3. The Cf values for the Cu, Zn and Ni were 0.41, 0.34 and 0.05 respectively, indicates 'low contamination factor' (Cf < 1; [26]). However, Pb showed Cf value of 1.52, indicates 'moderate contamination factor' ($1 \leq Cf < 3$; [26]). The plantation area was far away from main roads and industrial activities. Thus, the area was less subjected to pollution. The value of Cf in the decreasing order was Pb > Cu > Zn > Ni.

The Er values for the four metals range from 0.25-7.58. According to Hakanson [26], the Er value of less than 40 indicates low potential ecological risk posed by the availability of those metals in the soil. The pattern for Er in this study was similar to Cf, i.e. Pb > Cu > Zn > Ni.

Table 3. Values of contamination factor (Cf), ecological risk (Er)
and potential ecological risk index (PERI) in the topsoils of guava plantation in Kluang

Heavy metals	Cf	Er
Cu	0.41	2.06
Zn	0.34	0.34
Ni	0.05	0.25
Pb	1.52	7.58
PERI		10.23

The PERI value was calculated at 10.2, indicates low ecological risk (PERI < 150; [26]). In Kluang's plantation area, the soil is acidic with pH of 3-4. According to Ang and Ng [21], plants absorbing the heavy metal in the soil condition under acidic when pH is less than 5.0. The plantation owner treated the soil with agricultural limestone to neutralize the acidic soil condition. Thus, the plant can be cultivated productively. The usage of chemical pesticides and fertilizers were only carried out once a year. In general, they practice a good and ecological type farming.

Overall, the metal concentrations from this study have yet to exceed the preindustrial reference levels [26, 29].

3.3 *Health risk assessment*

The health risk assessment was carried out in relation to its carcinogenic effects as well as non-carcinogenic effects based on calculation of EDI and THQ. Table 4 showed the values of EDI and THQ in the edible pulps of guava. The contribution of heavy metals to the EDI for all consumers followed the order of Fe > Cu > Zn > Pb > Ni.

Table 4. Estimated daily intake (EDI) (µg/kg/day) and target hazard quotient (THQ) (mg/kg) of heavy metals in the edible pulps of guava by average adults and children of 60 kg and 19.25 kg body weight respectively

		Cu	Zn	Ni	Pb	Fe	Cu	Zn	Ni	Pb	Fe
				EDI					THQ		
Adult (CR = 150)	ALC	7.15	2.83	0.73	1.18	12.28	0.179	0.009	0.036	0.235	0.018
	HLC	2.15	3.88	0.95	1.85	18.33	0.054	0.013	0.048	0.370	0.026
Adult (CR = 300)	ALC	14.30	5.65	1.45	2.35	24.55	0.358	0.019	0.073	0.235	0.035
	HLC	4.30	7.75	1.90	3.70	36.65	0.108	0.026	0.095	0.370	0.052
Children (CR = 50)	ALC	7.43	2.94	0.75	1.22	12.75	0.186	0.010	0.038	0.235	0.018
	HLC	2.23	4.03	0.99	1.92	19.04	0.056	0.013	0.049	0.370	0.027
Children (CR = 100)	ALC	14.86	5.87	1.51	2.44	25.51	0.371	0.020	0.075	0.235	0.036
	HLC	4.47	8.05	1.97	3.84	38.08	0.112	0.027	0.099	0.370	0.054

Note: ALC: Average level consumer; HLC: High level consumer; CR = consumption rate.

The THQ values for Cu, Zn, Ni, Pb and Fe were all below 1 for both adults and children. Yap, et al. [25] described that the value of THQ of more than 1 will indicate the level of exposure is higher than ORD, which means the daily consumption of the fruits within the level has high possibility to cause negative health effects to consumers. In general, the consumers will pose no risk if the value is below 1. Thus, the current result indicates that there should be no non-carcinogenic risks of all five metals if the fruit were consumed.

4. Conclusions

This study indicates that the levels of Cu, Zn and Pb found in the pulps, peels and seeds of guava were above the WHO stipulated limits. However, the study also concluded that the metal levels in the soils were lower than the safe limits set by WHO. The abundance level of metals in this fruit was in the order Fe > Cu > Zn > Pb > Ni. The *Cf* values for the heavy metals indicated low-to-moderate contamination factors. The *Er* values for the metals indicated a low potential ecological risk. Based on the THQ values we concluded that no potential health risk is involved in consuming the guava. The consumers are considered to be safe. Nonetheless, regular monitoring of heavy metals should be conducted to examine for any possible human health risk of heavy metals when the guavas are to be consumed. Such monitoring is required for the consumer well-being. These findings could be used as a reference for further study.

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Conflict of interest

The authors declare no competing financial interest.

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