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A preliminary study on heavy metals in green mustard *Brassica juncea*: A human health risk assessment

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ABSTRACT

Green mustard *Brassica juncea* were collected from Brinchang (Cameron Highland, Pahang, Peninsular Malaysia collected in September 2016) and Sikamat (Seremban, Negeri Sembilan, Peninsular Malaysia collected in September 2013). Metal concentrations ranges (mg/kg dry weight) for the edible leaves of *B. juncea* were Cu (4.80-5.06), Fe (36.8-139), Ni (0.50-0.80), Pb (0.30-1.60) and Zn (25.7-32.4) from all both sampling sites. As for health risk assessment, the values of target hazard quotient for all metals in this study were below 1.00 in both adults and children. The current results have reflected no non-carcinogenic risks from all metals through the intake of green mustard. Still, heavy metal monitoring should be carried out periodically in this consumable popular vegetable.

Key words : *Human health risk, Heavy metals, Green mustard*

Introduction

Indian mustard *Brassica juncea* (Family: Brassicacea) has been identified with the capacity to take up and accumulate heavy metals (Raskin *et al*., 1997). This species is has been known to be a good agent for phytoremediation of heavy metals (Singh and Fulekar, 2012). For example, *B. juncea* has been proven as a good phytoremediator of Pb and Cu in the contaminated soils (Rahman *et al*., 2013).

It has been reported that the main route of metals intake into human body is through the consumption of vegetables (Khan *et al*., 2008). The public concern has been arisen due to the wastewater irrigation to vegatables (Khan *et al.,* 2008; Girisha and Ragavendra, 2009; Wang *et al*., 2012; Islam *et al.,* 2016). Thus, continuous monitoring of heavy metals in *B. juncea* should be conducted.

Recently, the health risks of heavy metals from vegetable consumption has been widely studiedin Malaysia (Yaacob *et al.,* 2018a, 2018b; 2019; Yap *et al*., 2019a, 2019b, 2019c, 2020). This greatly shows the significance of metal monitoring in the commercially important vegetables in Malaysia. However, the detailed study on the human health risk of heavy metals in *B. juncea* from Malaysia, is still lacking in the literature.

The main aims of this study is to 1) determine the concentrations of heavy metals (Cu, Fe, Ni, Pb, and Zn) in *B. Juncea* from two farming areas of Peninsular Malaysia, and 2) assess the human health risks of Cu, Fe, Ni, Pb, and Zn from the consumption of these green mustards.

Materials and Methods

The vegetable *B. Juncea* was collected from Brinchang (Cameron Highland, Pahang, Peninsular Malaysia collected in September 2016) and Sikamat (Seremban, Negeri Sembilan, Peninsular Malaysia collected in September 2013) (Figure 1). The collected samples were stored in clean polyethylene bags before being transported to the laboratory. The identification of species in this study was based on the morphology and classification of *B. Juncea* obtained from the literatures (Chin and Yap, 1999; Prohens and Nuez, 2008a, 2008b).

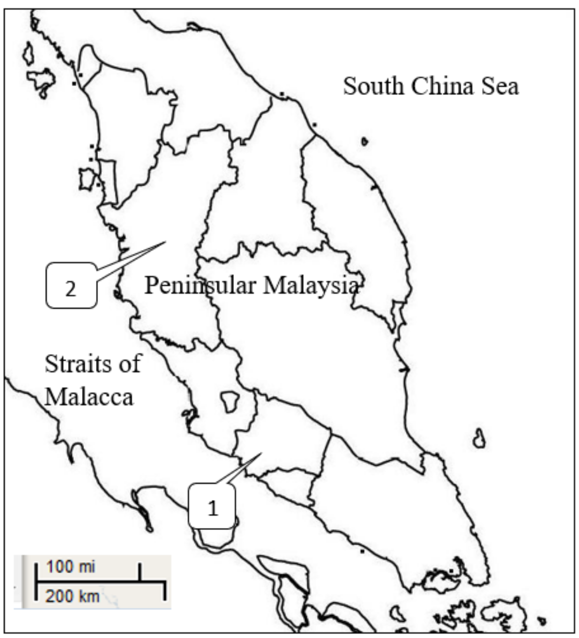

Fig. 1. The sampling sites for green mustard *Brassica juncea* in Peninsular Malaysia (1= Sikamat; 2= Brinchang).

In the laboratory, collected samples were washed and the leafy parts were cut into smaller sizes. Cut samples were then dried at 60 °C for 72 hours and grinded before they were kept in polyethylene bags.

The samples were then sent to the Chemistry Department of Faculty of Science at Universiti Putra Malaysia (UPM) for metal determination with the flame atomic absorption spectrophotometer (AAS) model Thermo Scientific iCE 3000. All data were recorded in mg/kg dry weight basis.

To avoid contamination, all the glassware in this study was acid-washed. The certified reference materials (CRMs) used in this study was *Lagarosiphon major* N.60 and Peach Leaves (NIST 1547). The analytical procedures and accuracy of the method used in this study were checked with the CRMs in which the recoveries obtained were optimal (*Lagarosiphon major* N.60: Zn = 97.4, Cu = 120.2, Pb = 119%; CRM Peach Leaves (NIST 1547):Ni = 97.0 and Fe = 117%).

As vegetables are consumed in fresh weight, the dry weight basis in this study were converted into wet weight basis by using CF for *B. juncea*. The human health risk assumes a once-or long-term potential hazardous exposure to metals through consumption of *B. juncea*. The assessment included estimated daily intake (EDI) and target hazard quotient (THQ) values were calculated by using the following formulas:

$$EDI = (Mc \times CR)/BW$$

where;

Mc= the metal concentration in vegetables (mg/kg wet weight).

CR= the consumption rate of vegetables (345 g/day for adults and 232 g/day for children) and average body weight (55.90 kg for adults and 32.70 kg for children) (Wang *et al.,* 2005).

The health risk assessment were measured based on the THQ, a non-carcinogenic risk assessment method based on the ratio of estimated dose of contaminant and oral reference dose (RfD). The THQ value below 1 indicated no appreciable risks. The formula for THQ is as follows (USEPA, 2000):

$$THQ = EDI / RfD$$

where; EDI= estimated daily intake; RfD= the oral reference dose (µg/kg wet weight/day) in which the values used were Cu: 40.0, Fe = 700, Ni = 20.0, and Zn: 300 (IRIS, 2000). The RfD for Pb was adopted from FAO/ WHO (2013), which is 4.00 µg/kg wet weight/day, as this data is not available in EPA's IRIS (2000). A value of THQ < 1 indicates that the vegetable consumption will result in non-carcinogenic risk of heavy metals to human health. The metal data for *B. Juncea* collected from Pearl River Estuary (China) and Chongqing (China) were cited from Li *et al.* (2012) and Yang *et al.* (2011), respectively. The EDI and THQ values of these reports were recalculated by using the formulae stated above.

Results and Discussion

Table 1 shows the heavy metal concentrations (mg/kg dry weight) of *B. juncea* (leafy part) collected from two farms in Peninsular Malaysia. For the edible leaves of *B. juncea*, the metal concentrations (mg/kg dry weight) for all samples ranged from 4.80-5.06 (Cu), 36.8-139 (Fe), 0.50-0.80 (Ni), 0.30-1.60 (Pb) and 25.7-32.4 (Zn) (Table 1). These present levels are within the ranges of Cu (0.47-19.6) and Pb (0.05-14.9), and higher than Ni (0.196) and Zn (3.28-42.4) (Table 1) as reported in the literature. When compared to the maximum permissible levels suggested by FAO/WHO (2011) (Cu: 40 mg/kg wet weight; Zn: 60 mg/kg wet weight) for leafy vegetables the Cu and Zn levels in *B. juncea*were lower in concentrations.

Kutrowska *et al.* (2017) found that metals influenced the distribution of Cu and Zn in *B. juncea* while Augusto *et al*. (2014) reported the effects of Cd and Pb contamination in nutrients causing drastic depletion of dry matter production in *B. juncea*.

Table 2 and 3 depicted the EDI and THQ values for all the metals in *B. juncea* for adults and children, respectively in the present study. It is shown that all THQ values for the five metals in both adults and children were below 1.00. The current data suggested that there are no non-carcinogenic risks of the five metals through the consumption of the *B. juncea* in this study. Islam *et al.* (2016) also reported the THQ values for Cu and Zn as were < 1.0 based on vegetables in Bogra District. Based on 30 veg-

Table 1. Mean heavy metal concentrations (mg/kg dry weight) in green mustard *Brassica juncea* collected from two farms in Peninsular Malaysia.

Site	Parts	Cu	Fe	Ni	Pb	Zn	SD	Source	Reference
Sikamat, Seremban.	stem Roots leaves	1.75 1.39 5.06	51.8 146 139	0.03 0.13 0.50	0.01 0.02 0.30	16.5 33.2 25.7	Sep-13	Wastewater for irrigation.	This study
Brinchang, Cameron Highland	Leaves	4.80	36.8	0.80	1.60	32.4	3-Sep-16	Roadside, cool temperate highlands, near farmer market area.	This study
Pearl River Estuary, China*	Leafy	0.47	NA	0.196	0.05	3.28	unspecified	Reclaimed tidal flat soil	Li *et al*. (2012)
Chongqing, China	leaves	19.6	NA	NA	14.9	42.4	2008-2009	27 super markets	Yang *et al.* (2011)

*= in wet weight basis. NA= data is not available. SD= sampling date.

Table 2. Values of estimated daily intake (EDI) of heavy metal concentrations in green mustard *Brassica juncea* collected from two farms in Peninsular Malaysia.

	Adults					Children				
Consumption rate of vegetables (g/day)	345					323				
Body weight (kg)	55.9					32.7				
	Cu	Fe	Ni	Pb	Zn	Cu	Fe	Ni	Pb	Zn
Seremban	2.85	78.57	0.28	0.17	14.47	4.57	125.75	0.45	0.27	23.17
Cameron	2.71	20.76	0.45	0.90	18.28	4.33	33.22	0.72	1.44	29.25
Pearl River Estuary, China	2.92	NA	1.21	0.29	20.24	4.67	NA	1.94	0.46	32.40
Chongqing, China	11.06	NA	NA	8.41	23.92	17.70	NA	NA	13.45	38.28

Note: All metal data were converted to wet weight basis using a conversion factor of 0.091 for the calculation of EDI.

Table 3. Values of target hazard quotient (THQ) of heavy metal concentrations in green mustard *Brassica juncea* collected from two farms in Peninsular Malaysia.

	Adults					Children				
RfD (µg/kg wet weight/day)	40.0 Cu	700 Fe	20.0 Ni	4.00 Pb	300 Zn	40.0 Cu	700 Fe	20.0 Ni	4.00 Pb	300 Zn
Seremban	0.071	0.112	0.014	0.042	0.048	0.114	0.180	0.023	0.068	0.077
Cameron	0.068	0.030	0.023	0.226	0.061	0.108	0.047	0.036	0.361	0.098
Pearl River Estuary, China	0.073	NA	0.060	0.073	0.067	0.117	NA	0.097	0.116	0.108
Chongqing, China	0.276	NA	NA	2.101	0.080	0.442	NA	NA	3.363	0.128

etables from Kunming City for seven metals, Zhang *et al.* (2017) reported the THQ values > 1.0 for adolescents, showing non-carcinogenic risks of the metals to the adolescents.

Conclusion

Generally, the current study suggested that the content of all five metals (both adult and children) in the *B. juncea* showed not non carcinogenic (THQ<1.0) risks from consumption. A check on the THQ values of the toxic metals are much needed from time to time on this popular vegetable to safeguard the public health from the possible metal toxicological impacts.

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