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Heavy Metals in Green Mustard *Brassica oleracea* : A human health Risk Assessment on The Edible Leaves

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Abstract

Green mustard *Brassica oleracea* were collected from Brinchang (Cameron Highland, Pahang, Peninsular Malaysia collected in 3 September 2016) and Tanah Tinggi Lojing (Gua Musang, Kelantan, Peninsular Malaysia collected in 6 August 2016). For the edible leaves of *B. oleracea*, the ranges of metal concentrations (mg/kg dry weight) from the three sites were Cu (6.80-14.0), Fe (41.6-102), Ni (1.20-1.21), Pb (0.80-2.00) and Zn (1.60-18.0). For the health risk assessment, all the target hazard quotient values for Fe, Cu, Ni, Pb, and Zn in both adults and children were found <1.00. This indicated that there were no non-carcinogenic risks of Fe, Cu, Ni, Pb, and Zn via the consumption of green mustard from the present study. Still, heavy metal monitoring should be carried out periodically in this consumable popular vegetable.

Keywords: Human health risk; Heavy metals; Vegetable

Introduction

Brassica oleracea is also known as green mustard or mustard leaf and is belonging to the family Brassicacea.. Zelano et al. [1] evaluated the possibility to use *B. oleracea* var. Acephala (kale) as a vegetal monitor for metals dispersed in the atmosphere. According to Mourato et al. [2], several species from the Brassica genus (including *B. oleracea*) are very important agricultural crops in different parts of the world and are also known to be heavy metal accumulators. Ahmad et al. [3] reported significant differences between the levels of the metals in *B. oleracea* collected from different urban farming sites in Sargodha, Pakistan. The public concern has been arisen due to the wastewater irrigation to vegetables [4-7].

In Malaysia, the human health risks of heavy metals in vegetables have been recently reported [8-14]. 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. oleracea* from Malaysia. is still lacking in the literature.

The objectives of this study were to

I. Assess the concentrations of Fe, Cu, Ni, Pb, and Zn in green mustard *B. oleracea* from two farming areas of Peninsular Malaysia, and

II. Assess the human health risks of Fe, Cu, Ni, Pb, and Zn in the collected green mustard.

Materials and Methods

Green mustard *B. oleracea* were collected from Brinchang (Cameron Highland, Pahang, Peninsular Malaysia collected in 3 September 2016) and Tanah Tinggi Lojing (Gua Musang, Kelantan, Peninsular Malaysia collected in 6 August 2016) (Figure 1). The collected samples were stored in clean polyethylene bags and transferred to the laboratory for further analyses. The morphology and classification of *B. oleracea* from the present study had been identified according to Chin & Yap [15], and Prohens & Nuez [16,17].

Figure 1: The sampling sites for *Brassica oleracea* in Peninsular Malaysia (1= Gua Musang; 2= Brinchang).

The collected samples were washed with distilled water to remove soil particles. Then, the leafy parts were cut into small pieces using a clean knife and were dried in an oven at 60 °C for 72 hours days until constant dry weights. After drying, the vegetable samples were grinded by using a commercial blender and stored in polyethylene bags until further analysis.

For determination of heavy metals, all samples stored in acid-washed pillboxes were analyzed by using a flame atomic absorption spectrophotometer (AAS) model Thermo Scientific iCE 3000 series for Fe, Cu, Ni, Pb, and Zn at Chemistry Department of Faculty of Science at Universiti Putra Malaysia (UPM). Standard solutions were prepared from 1000ppm stock solution provided by Sigma-Aldrich for the five metals. All data obtained from the AAS were presented in mg/kg dry weight basis.

For quality assurance and quality control, all the glassware used in this study were acid-washed to avoid external contamination. Two certified reference materials (CRMs) were used to check for the analytical procedures and accuracy of the method used. The CRMs included were Lagarosiphon major N.60 and Peach Leaves (NIST 1547). The recoveries for the CRM Lagarosiphon major N.60 were 97.4, 120.2, 119% for Zn, Cu and Pb, respectively, while CRM Peach Leaves (NIST 1547) were 97.0 and 117% for Ni and Fe, respectively (Table 1).

Table 1: Comparison of metal concentrations (mg/kg dry weight) between certified and measured values. The certified values are based on certified reference materials were Lagarosiphon major N.60 and Peach Leaves (NIST 1547).

	Certified Value	Measured Value	Recovery (%)
	Peach Leaves (NIST 1547)		
Fe	219.8	211	97
Ni	0.689	0.81	117
Lagarosiphon major (N.60)			
Cu	51.20 ± 1.9	61.54 ± 1.4	120.2
Pb	64 ± 4.00	76.3 ± 2.40	119
Zn	313 ± 8.0	304.85 ± 3.4	97.4

For the human health risk assessment, the present concentrations in dry weight basis were converted into wet weight basis because consumption (or cooking) of the vegetables are assumed to be in fresh weight. Therefore, the present concentrations (mg/kg dry weight) of Fe, Cu, Ni, Pb, and Zn were converted to wet weight basis by using CF for *B. oleracea*.

The human health risk assumes a once-or long-term potential hazardous exposure to metals through consumption of *B. oleracea*. The assessment included estimated daily intake (EDI) and target hazard quotient (THQ) values were calculated by using the following formulas:

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

where.

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

CR= the consumption rate of vegetables (345g/day for adults and 232 g/day for children) and average body weight (55.90 kg for adults and 32.70 kg for children) [18].

In this study, a non-carcinogenic risk assessment method was based on THQ, a ratio between the estimated dose of contaminant and the oral reference dose (RfD), below which there will not be any appreciable risk. The THQ was determined with a formula described by USEPA [19]:

$$THQ = EDI/ RfD$$

where.

EDI= estimated daily intake calculated previosuly;

RfD= the oral reference dose. The RfD (μg/kg wet weight/ day) values used in this study were Fe: 700, Ni: 20.0, Cu: 40.0, and Zn: 300, provided by the EPA's Integrated Risk Information System online database [20]. Since RfD for Pb was not available according to the EPA's IRIS [20], the present study employed the RfD as 4.00 μg/kg wet weight/day as proposed by FAO/ WHO [21]. It is estimated that if the THQ ratio is more than one (THQ < 1), the vegetable consumption will result in non-carcinogenic risk of heavy metals to human health. The calculation of EDI and THQ values, the metal data for *B. oleracea* collected from Pearl River Estuary (China) and Lahore (Pakistan) were cited from Li et al. [22] and Mahmood & Malik [24], respectively.

Results and Discussion

Concentrations of Fe, Cu, Ni, Pb, and Zn

The heavy metal concentrations (mg/kg dry weight) in the green mustard *B. oleracea* (leafy part) collected from two farms in Peninsular Malaysia are presented in Table 2. For the edible leaves of *B. oleracea*, the ranges of metal concentrations (mg/kg dry weight) from the three sites were Cu (6.80-14.0), Fe (41.6-102), Ni (1.20-1.21), Pb (0.80-2.00) and Zn (1.60-18.0) (Table 1). These present levels are within the ranges of Cu (0.74-37.7) and Pb (0.32-10.1), and lower than Ni (2.23-2.95) and Zn (4.40-80.7) (Table 1) as reported in the literature.

Table 2: Mean heavy metal concentrations (mg/kg dry weight) in edible leaves of *Brassica oleracea* collected from two farms in Peninsular Malaysia in comparison to those reported from the literature.

Site	Cu	Fe	Ni	Pb	Zn	SD	Source/background	Reference
Brinchang, Cameron Highland	14	102	1.2	0.8	1.6	3-Sep-16	Roadside, cool temperate highlands, near farmer market area.	This study
Tanah Tinggi Lojing, Gua Musang	6.8	41.6	1.21	2	18	6-Aug-16	Agricultural area surrounded by forest, highlands, distance from highway.	This study
Pearl River Estuary, China*	0.345 (3.83)	NA	0.201 (2.23)	0.074 (0.82)	2.31 (25.7)	Unspecified	Reclaimed tidal flat soil	Li et al. [22]
Varanasi, India	37.7	NA	NA	1.56	63.6	2004-2005	Market	Sharma et al. [23]
Varanasi, India	16.6	NA	NA	1.02	51.5	2004-2005	Production site	Sharma et al. [23]
Lahore, Pakistan	0.74	NA	2.95	0.32	4.4	Unspecified	Groundwater irrigated zone	Mahmood and Malik [24]
Lahore, Pakistan	4.49	NA	2.95	1.66	35.4	unspecified	Wastewater irrigated zone	Mahmood and Malik [24]
Chongqing, China	14.6	NA	NA	10.1	80.7	2008-2009	27 supermarkets	Yang et al. [25]

*= original data reported in wet weight basis. NA= data is not available. Values in brackets are those converted into dry weight basis by using a conversion factor of 11.1.

The differences in metal concentrations found in vegetable depended upon different soil nature and assimilation capacities of vegetables at different sites which in turn depended upon different environmental factors [26] such as temperature, moisture and wind velocity, and the nature of the vegetables [27].

The mean concentrations of Cu and Zn in *B. oleracea* from the three sites were lower than the maximum permissible levels suggested by FAO/WHO [28] (Cu: 40 mg/kg ww; Zn: 60 mg/kg ww) for leafy and fruit vegetables.

Health risk assessments

The values of EDI and THQ of the five heavy metals in *B. oleracea* for adults and children from the present study, are presented in Tables 3 & 4, respectively. All the THQ values for Fe, Cu, Ni, Pb, and Zn in both adults and children were found below

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1.00. This shows that there are no non-carcinogenic risks of the five metals through the consumption of the *B. oleracea* from the present study. Zhang et al. [29] reported that the THQ values based on 30 vegetables from Kunming City for seven metals was > 1.0 for adolescents. This indicated non-carcinogenic risks of heavy metals to the adolescents. Similarly, Islam et al. [7] also reported the THQ values for Cu and Zn as were < 1.0 based on vegetables in Bogra District.

Table 3: Values of estimated daily intake (EDI) of heavy metal concentrations in leaves of *Brassica oleracea* collected from three 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
Brinchang, Cameron Highland	7.78	56.7	0.67	0.44	0.89	12.5	90.68	1.07	0.71	1.42
Tanah Tinggi Lojing, Gua Musang	3.78	23.1	0.67	1.11	10	6.05	36.98	1.07	1.78	16
Pearl River Estuary. China*	2.13	NA	1.24	0.46	14.3	3.41	NA	1.99	0.73	22.8
Market, Varanasi, India	20.9	NA	NA	0.87	35.3	33.5	NA	NA	1.39	56.6
Production site, Varanasi, India	9.24	NA	NA	0.57	28.6	14.8	NA	NA	0.91	45.8
Groundwater irrigated zone, Lahore, Pakistan	0.41	NA	1.64	0.18	2.44	0.66	NA	2.62	0.28	3.91
Wastewater irrigated zone, Lahore, Pakistan	2.49	NA	1.64	0.92	19.69	3.99	NA	2.62	1.48	31.5
Chongqing, China	8.11	NA	NA	5.61	44.83	12.9	NA	NA	8.98	71.7

Note: All metal data were concerted to wet weight basis using a conversion factor of 0.09 for the calculation of EDI.
*= the data were not converted since the data is already in wet weight basis.
NA= data is not available.

Table 4: Values of target hazard quotient (THQ) of heavy metal concentrations in leaves of *Brassica oleracea* collected from three farms in Peninsular Malaysia

	Adults					Children				
	Cu	Fe	Ni	Pb	Zn	Cu	Fe	Ni	Pb	Zn
RfD (µg/kg wet weight/day)	40	700	20	4	300	40	700	20	4	300
Brinchang, Cameron Highland	0.194	0.081	0.033	0.111	0.003	0.311	0.13	0.053	0.178	0.005
Tanah Tinggi Lojing, Gua Musang	0.094	0.033	0.033	0.278	0.033	0.151	0.053	0.053	0.444	0.053
Pearl River Estuary. China*	0.053	NA	0.062	0.114	0.048	0.085	NA	0.099	0.183	0.076
Market, Varanasi, India	0.524	NA	NA	0.217	0.118	0.838	NA	NA	0.347	0.189
Production site, Varanasi, India	0.231	NA	NA	0.142	0.095	0.37	NA	NA	0.227	0.153
Groundwater irrigated zone, Lahore, Pakistan	0.01	NA	0.082	0.044	0.008	0.016	NA	0.131	0.071	0.013
Wastewater irrigated zone, Lahore, Pakistan	0.062	NA	0.082	0.231	0.066	0.1	NA	0.131	0.369	0.105
Chongqing, China	0.203	NA	NA	1.403	0.149	0.324	NA	NA	2.245	0.239

NA= data is not available.

Conclusion

For the health risk assessment, all the THQ values for Fe, Cu, Ni, Pb, and Zn in both adult and children are all below 1.00. This indicated that there were no non-carcinogenic risks of Fe, Cu, Ni, Pb, and Zn via the consumption of *B. oleracea* from the present study. Still, the present findings emphasized the need for regular monitoring of toxic heavy metals so as avoid contamination by the toxic metals on *B. oleracea* from the possible irrigation by using wastewater.

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References

1. Zelano V, Gulmini M, Grisello S, Torazzo A (2000) Use of Brassica oleracea var. Acephala as a vegetal monitor for metals in the atmosphere. Toxicological and Environmental Chemistry 78: 41-53.
2. Mourato MP, Moreira IN, Leitão I, Pinto FR, Sales JR, et al. (2015) Effect of heavy metals in plants of the genus Brassica. International Journal of Molecular Sciences 16(8): 17975-17998
3. Ahmad K, Khan ZI, Ashfaq A, Khan A, Shad HA, et al. (2016) Risk assessment of heavy metal and metalloid contamination on cabbage (*Brassica oleracea L.*) grown in wastewater-irrigated urban farming sites in sargodha, Pakistan. Philippine Agricultural Scientist 99(2): 186-190.
4. Khan S, Cao Q, Zheng YM, Huang YZ, Zhu YG (2008) Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. Environ Pollut 152(3): 686-692.
5. Girisha ST, Ragavendra VB (2009) Accumulation of heavy metals in leafy vegetables grown in urban areas by using sewage water and its effect, Archives of Phytopathology and Plant Protection 42(10): 956-959.
6. Wang Y, Qiao M, Liu Y, Zhu Y (2012) Health risk assessment of heavy metals in soils and vegetables from wastewater irrigated area, Beijing-Tianjin city cluster, China. J Environ Sci 24(4): 690-698.
7. Islam MS, Ahmed MK, Habibullah-Al-Mamun M (2016) Apportionment of heavy metals in soil and vegetables and associated health risks assessment. Stochastic Environ Res Risk Assess 30(1): 365-377.
8. Yaacob A, Yap CK, Nulit R, Omar H, Al-Shami SA, et al. (2018a) Assessment of health risks of the toxic Cd and Pb between leafy and fruit vegetables collected from selected farming areas of Peninsular Malaysia. Integr Food Nutr Metab 5(3): 1-9.
9. Yaacob A, Yap CK, Nulit R, Omar H, Al-Shami SA, et al., (2018b) A comparative study of health risks of Fe and Ni in the vegetables collected from selected farming areas of Peninsular Malaysia. J Aquat Pollut Toxicol 2(1): 21.
10. Yaacob A, Yap CK, Nulit R, Omar H, Aris AZ, et al., (2019) Health risks of essential Cu and Zn via consumption of vegetables and relationships with the habitat topsoils from three farming areas of Peninsular Malaysia. In: Soil Pollution: Sources, Management Strategies and Health Effects, (Ed) Chee Kong Yap, Nova Science Publishers, New York, USA. Chapter 9; Pp. 229-260.
11. Yap CK, Yaacob A, Ibrahim MH, Nulit R, Leow CS (2019a) Heavy metals in bitter gourd (Momordica charantia): Human health risk assessment. ARC Journal of Nutrition and Growth 5(1): 1-5.
12. Yap CK, Muhammad ZCZ, Rosimah N, Meenakshi N, Mohd HI, et al. (2019b) Heavy metals (Cu, Ni and Zn) in chili (Capsicum annuum) collected from selected farms in Selangor and their human health risk assessments. EC Nutrition 15(2): 01-06.
13. Yap CK, Yaacob A, Wong KW, Nulit R, Nallapan M, et al. (2019c) Human health risks of heavy metals in okra (Abelmochus esculentus) and lettuce (Lactuta sativa) collected from selected farms in Peninsular Malaysia. Food Sci Nutr Technol 4(3): 000180.
14. Yap CK, Cheng WH, Wong KW, Yaacob A, Razalai R, et al. (2020) Hlealth risks of essential Ni and Fe via consumption of water spinach Ipomoea aquatica collected from Peninsular Malaysia. Ann. Environ. Sci. Toxicol. 4(1): 001-004.
15. Chin HF, Yap EE (1999) Malaysian vegetables in colour: A complete guide. Kuala Lumpur: Tropical Press.
16. Prohens J, Nuez F (2008a) Vegetables I: Asteraceae, Brassicaceae, Chenopodicaceae, and Cucurbitaceae. New York: Springer.
17. Prohens J, Nuez F (2008b) Vegetables II: Fabaceae, Liliaceae, Solanaceae, and Umbelliferae. New York: Springer.
18. Wang X, Sato T, Xing B, Tao S (2005) Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. Sci Tot Environ 350(1-3): 28-37.
19. USEPA (United States Environmental Protection Agency) (2000) Risk-based Concentration Table. United State Environmental Protection Agency, Washington, DC, USA.
20. IRIS (Integrated Risk Information System) (2000) Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. US Environmental Protection Agency. (accessed 27.05.15).
21. FAO/WHO (2013) Guidelines for the Safe Use of Wastewater and food stuff; Volume 2: No1 14, pp 988. Wastewater Use in Agriculture, World Health Organization, Geneva.
22. Li Q, Chen Y, Fu H, Cui Z, Shi L, et al. (2012) Health risk of heavy metals in food crops grown on reclaimed tidal flat soil in the Pearl River Estuary, China. J Hazard Mater 227-228: 148-154.
23. Sharma RK, Agrawal M, Marshall FM (2009) Heavy metals in vegetables collected from production and market sites of a tropical urban area of India. Food and Chemical Toxicology 47(2009): 583-591.
24. Mahmood A, Malik RN (2014) Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan. Arabian J Chem 7(1): 91-99.
25. Yang QW, Xu Y, Liu SJ, He JF, Long FY (2011) Concentration and potential health risk of heavy metals in market vegetables in Chongqing, China. Ecotoxicology and Environmental Safety 74(6): 1664-1669.
26. Ahmad K, Ashfaq A, Khan ZI, Bashir H, Sohail M, et al. (2018) Metal accumulation in Raphanus sativus and Brassica rapa: an assessment of potential health risk for inhabitants in Punjab, Pakistan. Environmental Science and Pollution Research 25(17): 16676-16685.
27. Zurera G, Moreno R, Salmeron J, Pozo R (1989) Heavy metal uptake from greenhouse border soils for edible vegetables. J Sci Food Agric 49: 307-314.
28. FAO/WHO (2011) FAO/WHO. Joint FAO/WHO Food Standards Programme Codex Committee on Contaminants in Foods, Fifth Session, pp. 64-89.
29. Zhang H, Huang B, Dong L, Hu W, Akhtar MS (2017) Accumulation, sources and health risks of trace metals in elevated geochemical background soils used for greenhouse vegetable production in southwestern China. Ecotox Environ Saf 137: 233-239.

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