Health Risk Assessment of Heavy Metals in the Soursop (*Annona muricata*) from Kluang, Peninsular Malaysia

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Abstract. The present study studied the concentrations of Cu, Fe, Ni, Pb and Zn in the pulps, peels and seeds of soursop (Annona muricata) collected from Kluang (Johore, Peninsular Malaysia) and to estimate the human health risk of heavy metals in the edible soursop. It was found that the levels of Cu, Fe and Zn found in the pulps, peels and seeds of soursop were relatively low. Overall, the levels of Cu, Ni, Pb and Zn in the unripe pulps are significantly (P< 0.05) higher (except for Ni) than those in the ripe pulps. However, Fe level in the ripe pulp is significantly higher (P< 0.05) than that in the unripe pulp. The EF and Igeo for Pb indicated 'moderate enrichment' and 'unpolluted to moderately polluted', respectively. The target hazard quotient (THQ) values are all below 1.0 for Cu, Fe, Ni, Pb and Zn. This indicates that there are no non-carcinogenic risks of the five metals if the soursop pulps are to be eaten. We still recommend regular monitoring and human health risk assessment of heavy metals in the soursop.

Keywords: soursop, heavy metals, ecological risk, health risks.

Introduction

Soursop (Annona muricata) (Family: Annonaceae) is a native species from tropical areas of North and South America, although this species be found in Asia, Africa and American countries (Coria-Téllez et al., 2018: 662-691). Nutritionally, it is high in carbohydrate and a good source of vitamin B and C. As cited in Sun et al. (2009: 4382), annonaceous acetogenins in soursop fruit and other parts of the tree exhibit selective growth inhibition against cancer cell. Other part of the soursop tree are also used in natural medicine in the tropics, including the bark, leaves, roots, fruit, and fruit seeds to cool fevers, diarrhea and dysentery (Sun et al., 2009: 4382; Moghadamtousi et al., 2014: 15625). Several studies on different aspects have been studied with the soursop fruits; Coria-Téllez et al. (2018: 662-691) and Moghadamtousi et al. (2015: 15625) on traditional medicinal uses, Costa et al. (2014: 663) on its physicochemical characteristics, Passos et al. (2015: 66-73) on its glycemic index, Santana et al. (2017: 250-260) on its characteristic impact compounds, and Espinosa et al. (2013: 10-20) on its physiological and physicochemical behavior. However, the heavy metals in the edible fruits of soursop has yet to be widely explored. Recently, de Moraes et al. (2020: 676) reported the metal levels in A. muricata. Many papers documented the metal levels in the fruits (Waheed et al., 2004: 523-531; Radwan and Salama, 2006: 1273) and vegetables in Malaysia (Yaacob et al., 2018a: 1-9; 2018b: 21; 2019: 229-260; Yap et al., 2016: 285-296; 2019a: 1-5; 2019b: 1-6; 2019c: 000180; 2020: 1-4) except for edible soursop (de Moraes et al., 2020: 676) based on current literature. Since the information is lacking on the metal levels and its risk assessment, the objective of this study is to determine the concentrations of Cu, Zn, Ni, Pb and Fe in the pulps, peels and seeds of soursop collected from Kluang (Johore) and to estimate the human health risk of heavy metals in the edible soursop.

Materials and Methods

The sampling location for soursops was conducted on 1 December 2015, at Taman Kekal Pengeluaran Makanan (TKPM) (1°55′59.2428″ N; 103°11′8.8152″ E), Pusat Pertanian Ayer Hitam, Kluang, Johor approximately 300 KM from UPM Serdang. Samples of edible soursops were randomly collected which is unripe and ripe fruits. Fruits were collected from five individual plants (Table 1). The samples collected were put in polystyrene container and sort according to species and labelled systematically. The habitat topsoils collected were placed in sealable polyethylene plastic bag and kept in an iced box. All samples were transported to the laboratory and stored in freezer until further analysis.

Table 1. Description on measurement of soursop (*Annona muricata*) and its habitat topsoil collected from of soursop plantation in Kluang

Tree	Tree Height (m)	Fruit Description	Fruit diameter (cm)	Fruit Weight (g)	Soil Depth (cm)
A1	5-6	Ripe	40.55	675	10
A2	5-6	Unripe	22.00	230	10
A3	5-6	Unripe	26.00	320	10
A4	5-6	Unripe	21.70	157	10
A5	5-6	Ripe	30.50	400	10

The collected samples of soursops were washed with distilled water to remove dust particles, wiped with clean tissue paper and cut into small pieces with a stainless-steel knife. The soursop were separated into fleshy pulps, peels (skins) and seeds. The separated soursop parts were then put into aluminium foil and dried in the oven for 72 hours at 60°C to constant dry weight. The dried samples were crashed and using a pestle and mortar. After grinded, the soil samples were sieved through a 63 µm stainless steel sieve. All the plastic instruments and glassware were soaked in 10% of Nitric Acid solution mixed with 90% of distilled water. All the plastic instruments and glassware were rinsed through distilled water.

About 1.0g of the dried samples of peels, pulps and seeds of soursop were placed in digestion tubes and 10 mL of concentrated nitric acid (HNO_3; AnalaR grade; BDH 69%) was added. About 1.0 g of each dried topsoil 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. Three replicates were done for each fruit part and topsoil samples. The tubes were then put onto a hot block digester at 40°C for 1 hour and the contents were fully digested at 140°C for the next 3 hours. After that, the digested samples were diluted up to 40 mL with distilled water and then filtered through a Whatman No. 1 filter paper. The filtered solutions were collected and kept in acid washed pill boxes (Yap et al., 2016: 285-296).

After digestion via direct aqua regia method, the concentration of Cu, Fe, Ni, Pb, and Zn were determined by using an air acetylene flame atomic absorption spectrophotometer (AAS). The model used in this study was Thermo Fisher Scientific Spectrometer (S Series GE712405 v1.27). The data presented in mg/kg weight basis (Yap et al., 2016: 285-296). The recoveries of certified reference materials for MESS-3 were Cu (101%), Zn (57.7%), Ni (78.9%), Pb (107%) and Fe (53.9%).

Enrichment Factor (EF)

A common approach to estimate the anthropogenic impact on sediments is to calculate a normalized enrichment factor (EF) for metal concentrations above uncontaminated background levels The EF normalizes the measured concentration of heavy metal with respect to a sample reference metal such as Fe (Abrahim and Parker, 2008: 227-238). The calculation of EF based on Buat-Menerd and Chesselt (1979: 398-411):

$$\mathbf{EF} = \frac{\left(\frac{\mathbf{Me}}{\mathbf{Fe}}\right)\mathbf{Sample}}{\left(\frac{\mathbf{Me}}{\mathbf{Fe}}\right)\mathbf{Background}}$$

Where:

$(Me/Fe)_{Sample}$ refers to metal to Fe ratio in the sediments

$(Me/Fe)_{Background}$ refers to continental crust reference values (Cu= 25.0; Zn= 65; Ni= 56; Pb= 14.8 (Wedepohl, 1995: 1217-1232).

Index of geoaccumulation (Igeo)

Müller (1981: 157-164), propose the geoaccumulation index, Igeo to assess the degree of metal pollution based on seven classes according to the increasing numerical values of the index:

$$\text{Igeo} = \log_2\left(\frac{\text{Sample}}{1.5 \text{ x Background}}\right)$$

Where:

Sample refers to concentration measured in the soil;

Background refers to continental crust reference values (Cu= 25.0; Zn= 65; Ni= 56; Pb= 14.8 (Wedepohl, 1995: 1217-1232).

1.5 refers to correction factor due to lithogenic effluents.

Health risk assessment

The dry weight basis concentration of the heavy metals obtained previously were converted to wet weight basis by using conversion factor (ratio dry weight to wet weight of sample). According to Yen et al. (2011: 239-256), Malaysian average consumption of fruits and vegetables are 150.0 g and 78.0 g respectively. Food consumption between children and adult also differ. Children age range 0-6 years old with body weight 19.25 kg according to Guerra et al. (2010: 54-60). According to Yen et al. (2011: 239-256) for the Malaysian consumers, the consumption rate of fruits (general type of fruits) for average-level consumer (ALC) and high-level consumer (HLC) are 150 and 300 g/day, respectively for adult male. While for children, the consumption rate of fruits for ALC and HLC are 50.0 and 100 g/day, respectively.

In order to assess the health risks through consumption of soursop, the estimated daily intake (EDI) was calculated using the following formula (Cherfi et al., 2015: 245-252; Yap et al., 2016: 285-296):

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

Where:
Mc – metal concentration in wet weight basis (mg/kg)
CR – consumption rate for ALC and HLC (g/day)
ABW – average body weight 60 kg for adult (Yap et al., 2016: 285-296); 19.25 kg for children (Guerra et al., 2010: 54-60)

In order to assess the health risks through consumption of fruits in the two study areas, the target hazard quotient (THQ) was calculated using the following formula (Cherfi et al., 2015: 245-252; Yap et al., 2016: 285-296):

$$THQ = EDI/ORD$$

Where:
ORD – oral reference dose (µg/kg/day)

ORD (µg/kg/day) values used in the present study were: Cu: 40; Zn: 300; Ni: 20; and Fe: 700, as mentioned in USEPA's regional screening level (USEPA, 2015; Yap et al., 2016: 285-296). For Pb, the ORD is based on provisional tolerable daily intake as 3.57 (FAO/WHO, 2001).

If the value of THQ > 1, it indicates that 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 (Yap et al., 2016: 285-296; Khairiah, 2014: 307-312).

Results and Discussion

Metals in the soursop

Heavy metal concentrations in the pulps, peels and seeds of soursop and its habitat topsoils collected from of soursop plantation in Kluang are presented in Table 2.

Table 2. Heavy metal concentrations (mg/kg dry weight except for those indicated *) in the pulps, peels and seeds of soursop (*Annona muricata*) and its habitat topsoils collected from of soursop plantation in Kluang

Part		Cu	Zn	Ni	Pb	Fe
Pulps	Ripe	11.0 ± 0.86	7.25 ± 0.32	3.59 ± 0.60	0.97 ± 1.31	65.2 ± 15.7*
	Unripe	35.4 ± 5.60*	23.8 ± 5.47*	3.96 ± 0.76	3.83 ± 0.96*	45.2 ± 2.21
Pulps**	Ripe	2.20	1.45	0.72*	0.19	13.1*&
	Unripe	3.54*	2.38*	0.40	0.38*	4.52
Peels	Ripe	8.53 ± 0.42	8.68 ± 0.27	3.36 ± 0.22	2.86 ± 0.94	48.5 ± 2.20
	Unripe	10.3 ± 0.32	10.3 ± 0.90	3.18 ± 0.61	2.57 ± 0.31	45.4 ± 1.28
Seeds	Ripe	22.6 ± 0.70	17.7 ± 1.04	5.19 ± 0.22	2.41 ± 1.80	54.1 ± 2.34

	Unripe	27.8 ± 5.30*	30.5 ± 7.47*	8.06 ± 0.92*	3.58 ± 3.17*	70.2 ± 3.74*
Topsoils		18.3 ± 1.15	25.8 ± 0.41	2.24 ± 0.69	36.3 ± 2.47	31276 ± 2466
Note: **= wet weight basis. The conversion factors used were 0.10 and 0.20 for unripe and ripe soursop pulps, respectively. *= significantly (P< 0.05) higher.						

The metal concentrations (mg/kg dry weight) in the edible ripe pulps are Cu (11.02), Zn (7.25), Ni (3.59), Pb (0.97) and Fe (65.2) while in the unripe pulps are Cu (35.4), Zn (23.8), Ni (3.96), Pb (3.83) and Fe (45.2).

Nascimento et al. (2020: 508-516) investigated frozen soursop pulps from the southern region of the state of Bahia, Brazil, and reported the range of concentrations (mg/kg wet weight) as Cu (0.30-0.88), Zn (0.36-0.84) and Fe (1.40-4.05). The levels are lower than the present study in which the concentrations (mg/kg wet weight) for the ripe soursop of Cu, Zn and Fe are 2.20, 1.45 and 13.1, respectively.

Overall, the levels of Cu, Zn, Ni and Pb in the unripe pulps are significantly (P< 0.05) higher (except for Ni) than those in the ripe pulps. However, Fe level in the ripe pulp is significantly higher (P< 0.05) than that in the unripe pulp. For peels, the levels of Cu, Zn, Ni, Pb and Fe are not significantly (P> 0.05) different between ripe and unripe soursops. For seeds, the levels of of Cu, Zn, Ni, Pb and Fe in the unripe seeds are significantly (P< 0.05) higher than those in the ripe seeds. The different levels of metals between ripe and unripe parts of soursop could be due to differences in water content and the maturity stage of the tree that bear the fruits (Li et al., 2007: 405-412).

The trend of distribution of heavy metal levels in the ripe soursop's flesh (pulps) is Fe>Cu>Zn>Ni> Pb. The metals present in fruit's pulp were dominated by Fe, Cu, and Zn as these nutrients play major roles in optimal plant growth and major enzymatic activities (Hopkins, 1999). Zn goes about as a significant activator of proteins in integrating the hormone forerunner (Maschner, 1986) while Fe is a constituent in chlorophyll that partaking in electron transport in the photosystem(Hopkins, 1999). As indicated by Radwan and Salama (2006: 1278), the levels of Cu and Zn were the most elevated when contrasted with Pb in different organic products species inspected from nearby market in Alexandria city (Egypt).

EF and Igeo

The values of EF and Igeo in the habitat topsoils from Kluang are presented in Table 3.

Table 3. The values of enrichment factor (EF) and geoaccumulation index (Igeo)of heavy metals in the habitat topsoils of soursop plantation in Kluang

Cu		Zn		Ni		Pb	
EF	Igeo	EF	Igeo	EF	Igeo	EF	Igeo
0.71	-1.86	0.58	-2.14	0.08	-4.93	2.60	0.02

The values of EF are 0.71 for Cu, 0.58 for Zn, 0.08 for Ni and 2.60 for Pb. According to Buat-Menerd and Chesselt (1979: 398-411), they are categorized as 'deficiency to minimal enrichment' (EF< 2.0), indicating Cu, Zn, and Ni are not present in extreme concentration or the soil are not too enriched with the metals. However for Pb, it is categorized as 'moderate enrichment' (EF= 2.0-5.0). EF values for metals were generally in the order of Pb>Cu>Zn>Ni. The pattern for EF in this study was Pb>Cu>Zn, while in previous study by Cheng and Yap (2015: 156-165) was Pb>Zn>Cu.

Igeo values for Cu, Zn, Ni and Pb in Kluang are -1.86 for Cu, -2.14 for Zn, -4.93 for Ni and 0.02 for Pb. The Igeo values for Cu, Zn and Ni are <0, which is categorized as 'unpolluted' while Pb Igeo is categorized as 'unpolluted to moderately polluted' (Igeo 0-1) (Müller, 1981: 157-164).

Health risk assessment

The EDI and THQ values of heavy metals in the edible pulps of soursops are presented in Table 4.

Table 4. Estimated daily intake (EDI) and target hazard quotient (THQ) of heavy metals (mg/kg wet weight) in the edible pulps of soursop (*Annona muricata*) collected from of soursop plantation in Kluang

		Cu	Zn	Ni	Pb	Fe	Cu	Zn	Ni	Pb	Fe
		EDI					THQ				
	Oral reference dose	-	-	-	-	-	40.0	300	20.0	3.57	700
ALC	Adult= 60kg	5.50	3.63	1.80	0.48	32.63	0.138	0.012	0.090	0.133	0.047
HLC	CR= 150	8.85	5.95	1.00	0.95	11.30	0.221	0.020	0.050	0.266	0.016
ALC	Adult= 60kg	11.00	7.25	3.60	0.95	65.25	0.275	0.024	0.180	0.266	0.093
HLC	CR= 300	17.70	11.90	2.00	1.90	22.60	0.443	0.040	0.100	0.532	0.032
ALC	Children=19.25kg	5.71	3.77	1.87	0.49	33.90	0.143	0.013	0.094	0.138	0.048
HLC	CR= 50	9.19	6.18	1.04	0.99	11.74	0.230	0.021	0.052	0.276	0.017
ALC	Children=19.25kg	11.43	7.53	3.74	0.99	67.79	0.286	0.025	0.187	0.276	0.097
HLC	CR= 100	18.39	12.36	2.08	1.97	23.48	0.460	0.041	0.104	0.553	0.034

Note: The conversions to wet weight basis are based on conversion factors of 0.12 and 0.19 for ripe pulp and unripe pulp, respectively. ALC: Average level consumer; HLC: High level consumer; CR= consumption rate.

The THQ values are all below 1.0 for Cu, Zn, Ni, Pb and Fe. This shows that there are no non-carcinogenic risks of the 5 metals if the soursops pulps are to be consumed. Ikechukwu et al. (2019: 39-45) studied THQ of 9 heavy metals in selected fruits sold at Umuahia market in Nigeria, including the soursop. They found all the THQ values were below 1.0 with no adverse effects on health of consumers.

Conclusion

This study demonstrated the degrees of Cu, Fe and Zn found in the pulps, strips and seeds of soursops were moderately low. Overall, the levels of Cu, Zn, Ni and Pb in the unripe pulps are significantly (P< 0.05) higher (except for Ni) than those in the ripe pulps. However, Fe level in the ripe pulp is significantly higher (P< 0.05) than that in the unripe pulp. The EF and Igeo for Pb indicated 'moderate enrichment' and 'unpolluted to moderately polluted', respectively. The THQ values are all below 1.0 for Cu, Fe, Ni, Pb and Zn. This shows that there are no non-carcinogenic risks of the five metals if the soursops pulps are to be consumed. Regardless, normal observing of hefty metals ought to be led to check for any conceivable human wellbeing danger of weighty metals when the soursops are to be burned-through.

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