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Heavy Metals (Cu, Ni and Zn) in Chili (*Capsicum annuum*) Collected from Selected Farms in Selangor and their Human Health Risk Assessments

Chee Kong Yap[1]*, Muhammad Zulkelfli Che Zaid[1], Rosimah Nulit[1], Meenakshi Nallapan[1], Mohd Hafiz Ibrahim[1] and Chee Seng Leow[2]

[1]*Department of Biology, Faculty of Science, University Putra Malaysia, Serdang, Selangor, Malaysia*

[2]*Humanology Sdn Bhd, Kuala Lumpur, Malaysia*

***Corresponding Author:** Chee Kong Yap, Department of Biology, Faculty of Science, University Putra Malaysia, Serdang, Selangor, Malaysia.

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Abstract

In this study, the concentrations of Cu, Ni and Zn were determined in the chili *Capsicum annuum* (Family: Solanaceae) collected from selected farms in Selangor. The metal concentrations (µg/g dry weight) ranged from 4.38 - 10.8 for Cu, 13.7 - 17.0 for Ni, and 34.7 - 118 for Zn. For human health risk assessments, the target hazard quotient (THQ) values of Cu, Ni and Zn are below than 1.0, indicating that the chili in those six farms causes no non-carcinogenic risk to the consumers. Therefore, these chilies are safe to be consumed.

Keywords: *Heavy Metals; Capsicum annuum; Selangor*

Introduction

Vegetables grown at contaminated sites could take up and accumulate metals at toxic concentrations [1]. Chili plant *Capsicum annuum* is the family of Solanaceae that is same with the potatoes, tomatoes and eggplant are a very famous home plant that been cultivated. In Malaysia, chili is very important vegetable on the basis of commercial value. Chili has economic and nutritious importance. According to a review by Limmatvapirat., *et al*. [2] *C. annuum* is a popular spice in Thailand.

Heavy metal studies in the edible *C. annuum* can be widely found in the literature. For examples, heavy metal levels in *C. annuum* have been reported from Kosovo [3], Nigeria [4], Pakistan [5], Malaysia [6], Thailand [2] and Algeria [7,8].

Antonious [9] reported the concentrations of seven metals (Cd, Pb, Ni, Mo, Cu, Zn, and Cr) in the fruits, leaves, stem, and roots of *C. annuum* plants grown under four soil management practices. Zeinullahu [3] evaluated the heavy metal content of As, Cr, Ni, Pb and Zn in *C. annuum* because the plants serve as the absorption system of these metals. Bhutto., *et al*. [5] assessed the effect on heavy metal concentration in fruits of major variety of C. annuum between field and market samples. Waziri and Adamu [4] showed contamination of agricultural chilli by Ni, Cd and Pb.

Hbaiz., *et al*. [10] investigated the effect of sludge on the various agronomic parameters measured and the toxicological impact of heavy metals in different organs in *C. annuum* Hbaiz., *et al*. [11] showed that the use of wastewater in the irrigation intensifies the levels of heavy metals in organs of the *C. annuum*. Accumulation of Cu and Zn is one of the constraints to this development. Limmatvapirat., *et*

al. [2] determined the concentrations of eleven heavy metals in *C. annuum* of fresh fruits collected from nine provinces located in the west of Thailand. They found that the concentrations of Cu and Zn were less than the permissible limits (20 and 100 mg/kg, respectively). Li., *et al*. [12] sampled whole *C. frutescens* plants and root soils from capsicum fields in three villages in the suburbs of Guiyang, Southwest China. They found that that Cd was over proof in the capsicum fruit.

Objective of the Study

The objective of this study was to determine the background concentrations of Cu, Ni and Zn in six selected farms of *C. annuum* from Selangor and to assess their human health risks.

Materials and Methods

Samples were collected from six different places, between December 2017 - January 2018, in Selangor as shown on figure 1 namely University Putra Malaysia (UPM) (Ladang 10), Ijok, Sabak Bernam, Selangor, Taman Kekal Pengeluaran Makanan (TKPM) at Bukit Changgang (two populations), and Agrotek. These farm sites were selectively chosen because most of their chili supplies are marketed within Klang valley in Selangor.

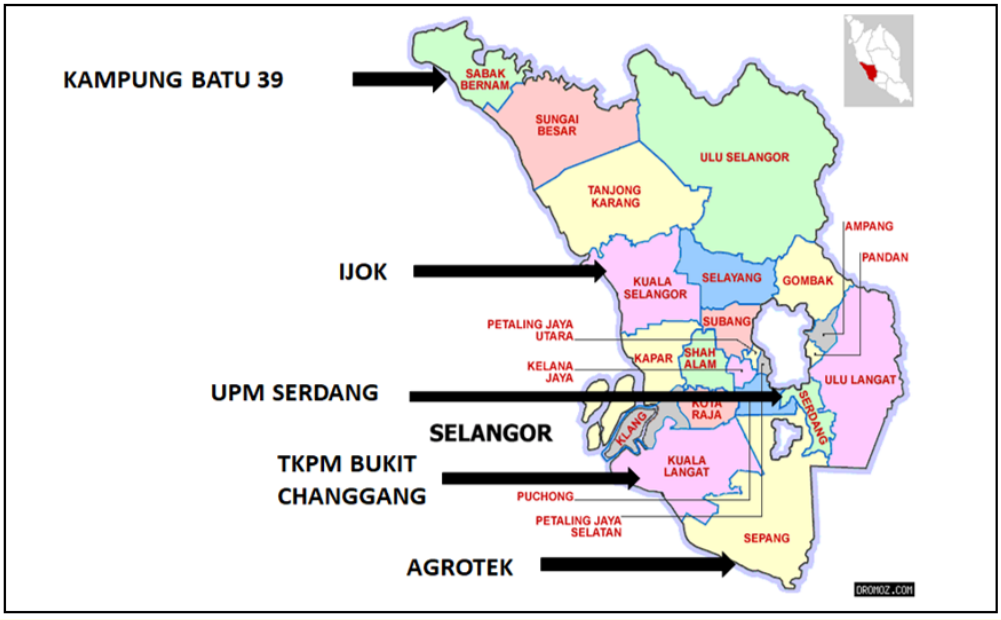

Figure 1: *Map showing the sampling sites at (A) Sabak Bernam, (B) Ijok, (C) UPM, (D) TKPM Bukit Changgang, and (E) Agrotek.*

Samples are clean under the tap water and then washed with distilled water. Then the chili is cut into small pieces (cube) like shown in figure 1 for better digestion. The chili is not separate by parts because of the chili usually be consumed with their seed as raw or in product like chili sauce.

The samples were dried in an oven for three days at 80°C. The dry weight of the sample is needed to calculate the conversion factor. For better homogeneity, the sample than been crushed using the mortar and pestle [13].

About 0.5g of sample from each site balanced on the balancer before been put into the acid wash digestion tube. For the digestion process, the samples were digested in 10 ml of nitric acid. All the process involving the chemical need to be done under the chamber for safety. The digestion tube has been put on the digestion block and they were heated for 40°C for one hour before the temperature been increased to 140°C for three more hours. The digested sample was diluted in 40ml of distilled water before cooled down, as used by Yap., *et al* [13].

Then, the samples were filtered through Whatman No1 filter paper into the pillboxes. The concentrations of Cu, Ni and Zn will be determined by using an air-acetylene flame atomic absorption spectrophotometer.

For quality control and quality assurance, the samples were rinsed with distilled water for purification. Then, the apparatus used was acid-washed with 10% diluted hydrochloric acid for at least 24 hours before used. The blank solution also was treated and digested at the same time. The blank solution was prepared to prevent from any metal contamination of the solution used. The standard solution prepared was analyzed first before sample analysis to determine the limit of the concentration. Besides, the data collected were converted to unit of ppm and compared to the Certified Reference Materials values to certificate the data. The CRM materials used were MESS-3 for topsoil habitat and lagarosiphon major for fruit sample, as shown in table 1.

Table 1: *Analytical results for certified reference materials and measured values for Cu, Ni and Zn.*

Metal	Samples	CRM (ppm) (mean ± Std)	Measured value (ppm) (mean ± Std)	Percentage of recoveries (M/C x 100%)
Cu	MESS-3 (marine sediment)	34 ± 1.6	27.988 ± 0.935	82.317
	LAGAROSIPHON MAJOR NR.60 (Community Bureau of Reference)	51.2 ± 1.9	42.910 ± 0.680	83.81
Ni	MESS-3 (marine sediment)	46.9 ± 2.2	33.244 ± 0.253	70.882
	LAGAROSIPHON MAJOR NR.60 (Community Bureau of Reference)	NA	NA	
Zn	MESS-3 (marine sediment)	159 ± 8	126.169 ± 1.108	79.352
	LAGAROSIPHON MAJOR NR.60 (Community Bureau of Reference)	313 ± 8	263.509 ± 2.1251	84.12

The conversion factor function is to determine the concentration of heavy metals in wet weight as the concentration of heavy metals intake by converted it from dry weight of the flesh Conversion factor = Dry Weight/Fresh Weight.

Human health risk assessment

The estimated daily intake is to calculate how much of chili that be taken by an adult for one day. First, the dry weight (dw) basis was converted to wet weight (ww) by using the conversion factor (0.09) as follow:

Wet weight (WW) = metal concentration (MC) X conversion factor.

The mean concentrations of the samples are needed for calculation of estimated daily intake of chili. The estimated daily intake (EDI) (µg/kg/day) of chili that contain of heavy metal element of Cu, Ni and Zn were measured by using a formula:

EDI= MC X CR/(BW).

MC represents the metal concentration (µg/g wet weight) in the chili. The body weight (BW; kg) for adults is 62 kg and consumption rate (CR; g/person/day) for fruit vegetables is 32g, following the report for Selangor population [14].

The human health risk assessment of the heavy metal in the guava fruits was determined by the calculation of Target Hazard Quotient (THQ). If the THQ value is higher than 1.0, this means that the daily consumption of chili would likely result in negative health effects during a lifetime in a human population [15]. The formula of THQ calculation was described as follow:

THQ= EDI/RfD

RfD represents the oral references dose (µg/kg/day). The reference doses used for Cu, Ni, and Zn are 40, 20 and 300, respectively, provided by the USEPA's regional screening level [16].

Results and Discussion

The concentrations of Cu, Ni and Zn in chili of 6 selected farms in Selangor are presented in table 2. Overall, the metal concentrations (µg/g dry weight) ranged from 4.38 - 10.8 for Cu, 13.7 - 17.0 for Ni, and 34.7 - 118 for Zn. TKPM is divided by two sub-sites (two populations) because of the large area of plantation.

Table 2: *Concentration (µg/g) of Cu, Ni and Zn) of Capsicum annum collected from 6 selected farm in Selangor. DW: Dry weight; WW: Wet weight.*

		Cu	Ni	Zn		Cu	Ni	Zn
No.	**Site**	**DW**	**DW**	**DW**	**CF**	**WW**	**WW**	**WW**
1	TKPM 1	4.38	13.7	38.4	0.09	0.40	1.25	3.49
2	TKPM 2	7.50	15.3	34.7	0.09	0.68	1.39	3.16
3	UPM	10.8	16.6	35.2	0.09	0.98	1.51	3.20
4	Sabak Bernam	5.51	17.0	59.3	0.09	0.50	1.55	5.40
5	Agrotek	7.20	16.1	118	0.09	0.66	1.47	10.7
6	Ladang	6.23	16.8	62.4	0.09	0.57	1.53	5.68

Note: *TKPM: Taman Kekal Pengeluaran Makanan; CF: Conversion Factor.*

The differences of metal levels among the sampling sites could be due to chemical factors. The use of traditional fertilizers and pesticides that contain chemicals, causing an increase in heavy metal uptake by chili plants. Agrotek uses chemical fertilizer to enhance the production of chili. It is to fulfil the demand of chili in the market. In TKPM, they use an organic fertilizer that been mix in the water to water the plant. Besides, in TKPM, the use of water from an abandoned mine could be the source. For Sabak Bernam site, they use the water from the pipe to water the plant. However, these observable non-point sources of pollution are uncertain, and only further studies can confirm the potential causes. Villarreal Núñez., *et al*. [17] concluded that the intensive use of agrochemistry associated the high soil losses by erosion could determine severe risks of contamination of crop *C. annum* at State of Rio de Janeiro, Brazil.

Values of EDI and THQ for Cu, Ni and Zn in *C. annum* are given in table 3. Overall, the values of EDI ranged from 0.64 - 0.80 for Ni, 0.21 - 0.51 for Cu, and 1.63 - 5.54 for Zn. The values of THQ for Cu, Ni and Zn are all below 1.0. The THQ value for all six selected farm in Selangor shows that the value of it is less than 1. This indicated that the chili populations from the six sites are safe for consumption at least based the sampling period.

Table 3: *Values of estimated daily intake (EDI) and target hazard quotient (THQ) for Cu, Ni and Zn in Capsicum annum collected from 6 selected farm in Selangor.*

Site	BW (kg)	CR (g/person/ day)	EDI (µg/kg/day)			RfD (µg/kg/day)			THQ		
		Ni	Cu	Ni	Zn	Ni	Cu	Zn	Cu	Ni	Zn
TKPM 1	62	32	0.21	0.64	1.80	20	40	300	0.005	0.032	0.006
TKPM 2	62	32	0.35	0.72	1.63	20	40	300	0.009	0.036	0.005
UPM	62	32	0.51	0.78	1.65	20	40	300	0.013	0.039	0.006
Sabak Bernam	62	32	0.26	0.80	2.79	20	40	300	0.006	0.040	0.009
Agrotek	62	32	0.34	0.76	5.54	20	40	300	0.008	0.038	0.018
Ladang	62	32	0.29	0.79	2.93	20	40	300	0.007	0.039	0.010

Note: *CR: Consumption Rate; BW: Body Weight; RfD: Oral Reference Dose; EDI: Estimated Daily Intake; THQ: Target Hazard Quotient.*

Conclusion

This study investigated the background concentrations of Cu, Ni and Zn in *C. annuum* collected from 6 selected farms in Selangor. Overall, the metal concentrations (µg/g dry weight) ranged from 4.38 - 10.8 for Cu, 13.7 - 17.0 for Ni, and 34.7 - 118 for Zn. For human health risk assessments, the THQ values of Cu, Ni and Zn are below than 1.0. These values indicated that the edible chilies collected from these 6 farms from Selangor would cause no non-carcinogenic risk of Cu, Ni and Zn to the consumers. Therefore, these chilies are safe to be consumed from the present study.

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