

# The Transfer Factor of Cadmium in Fern Leaves and Its Potential Health Risk

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## ABSTRACT

**Objective:** This cross sectional study was to model the soil-plant transfer mechanism of Cd in fern leaves and the health risk associated.

**Method:** A total of 29 fern leaves and soil were sampled from several locations in oil palm plantation and wet market in Pontian, Johor, between June to September 2015. Acid digestion method and Na<sub>2</sub>EDTA solution were used to digest the sample and the Cd concentration were analyzed using Atomic Absorption Spectrophotometer (AAS). The soil-plant transfer mechanism was calculated by Transfer Factor quotient (TF) and human health risk was assessed by Target Hazard Quotient (THQ).

**Results:** In general, Cd was higher in red fern leaves ( $2.26 \pm 0.06$  µg/kg) compared to the green fern ( $0.48 \pm 0.10$  µg/kg). Cd was also slightly higher in red fern sampling soil ( $3.32 \pm 0.03$  µg/kg) compared to green fern soil ( $2.87 \pm 0.005$  µg/kg) ( $Z = -2.402$ ,  $p = 0.016$ ). Cd in fern leaves and soil samples were within the acceptable range specified by the Malaysia Food Regulation 1985 ( $1 \times 10^3$  µg/kg) and the Dutch reference value ( $8 \times 10^2$  µg/kg). There was no significant correlation between Cd in soil with the fern leaves. Cd was highly accumulated in the roots of red fern leaves ( $0.71 \pm 0.60$  µg/kg). The soil-plant transfer mechanism indicated that TF was high in root-soil ( $0.98$  µg/kg) and shoot-soil ( $0.51$  µg/kg) of red fern. The THQ demonstrated that the reference population was within the acceptable of carcinogenic and non-carcinogenic health risk.

**Keywords:** *Cadmium, Fern leaves, Transfer Factor, Soil, Health Risk*

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## 1. Introduction

Cadmium (Cd) is a soft silver-white metal which can be found naturally in Earth's crust in its purest form. Cd is mobile in most of soil environment and the exposure occurrence normally through the food chain (Iñigo et al., 2013). The utilization of certain chemical fertilizers and pesticides contributes to accumulation of Cd in soil over time. These will eventually result in the Cd being taken up by surrounding plants (ATSDR, 1999; Liu et al., 2003; Sharma et al., 2007). Long term use of such chemical fertilizers has become a major concern (UNEP, 2006).

Cadmium is a highly toxic element that can cause serious systemic problem to human (Khan et al., 2009). Human cadmium exposure mostly comes from the consumption of Cd contaminated plants (Wang et al., 2006). The major pathway of human exposure to plants heavy metals is from soil-plant transfer mechanism (Cui et al., 2004; Bothe, 2011). Heavy metals from vegetable consumption may contribute to health risks (Munisamy et al., 2013). For instance, acute oral ingestion of Cd may results in severe gastroenteritis (ATSDR, 2008), while chronic effect of Cd toxicity may cause renal tubular dysfunction from Cd accumulation (WHO, 2010). Cd also may cause bone or skeletal changes (Jarup et al., 2000) that

appear next after renal tubular dysfunction, lung cancer (Sorhan and Esmen, 2004).

Green fern leaves or *Diplazium esculentum* is a vegetable fern with high antioxidant (AVRDC, 2014) mostly found in Asia region. The young and immature leaves can be eaten raw, boiled or stir-fried (Bandyopadhyay & Mukherjee, 2009). Fern leaves is in the family of *Athyriaceae* or *Dryopteridaceae* (Lai and Lim, 2011). It is vascular plants that reproduce via spores without seed or flowers. This plant has stems, leaves and fiddleheads that expand into fronds (McCausland and Jim, 2009). The average height of this plant is between 0.5 m to 1.5 m (Jasim et al., 2014). They grow in open forest gaps and cultivated area (Shalini et al., 2007). *Stenochlaenapalustris* is known as red fern leaves. Another type of ferns that is commonly consumed as vegetables. It belongs to the *Blechnaceae* family which are commonly found in the eastern tropics such as India, China, Malaysia and Australia. The young fronds are tender, edible but the old ones are very stiff and leathery (Holtum and Ridley, 1968). Red fern is one of the most common ferns found in oil palm plantation (Badrul, 2012). The common parts consumed are the young leaves and fronds (Liu et al., 2012). The fronds of *Helminthostachys zeylanica*, *Ceratopteris thalictroides*, *Diplazium esculentum* and *Stenochlaenapalustris* are eaten as salad and vegetables in Malaysia and Philippines (Yujing Liu, 2012).

There are few studies which determined the heavy metals such as Pb and Ni accumulated in fern leaves species of *Diplazium esculentum* (Pongthornpruek et al., 2008). Many other heavy metals such as Pb, Zn, Cu, Mn, and Cr also were highly detected in the roots of fern leaves compared to the fronds (Jasim et al., 2014). However, limited research done to determine the uptake of Cd in fern leaves. This study was to determine the transfer factor of Cd in fern leaves grow in area with high fertilizer usage and its potential health risk. The transfer factor of Cd from soil to plant was determined through transfer factor quotient (TF) while the health risk level from contaminated fern leaves consumption was measured through Target Hazard Quotient (THQ). This study fills in the gap of heavy metals uptake by fern leaves and the health risk through consumption of this plant especially in the tropical region.

## 2. Materials and Methods

This is cross-sectional study design conducted from June to September 2015. Fern leaves and soil samples were collected from oil palm plantation in Kampung Seri Bunian, Pontian, Johor (Figure 1). Pontian is the major palm oil,

pineapple and other food crops cultivation area in Southern part of Peninsular Malaysia. Nearly 25,000 hectares has been used for palm oil agriculture area (Association of Southeast Asian Nations, 1991). Types of soils present at the oil palm plantation in this area are peat and clay soil. Most of green fern leaves found in the oil palm plantation while the red fern leaves are easily found in the bushes near the oil palm plantation. Convenient sampling was used to collect fern leaves and soil samples.

Five samples of red and green fern leaves were taken from few sites of oil palm plantation in Pontian, Johor an extensive agriculture setting with high usage of pesticides and fertilizer reported in Malaysia. The utilization of chemical fertilizers contributes to accumulation of Cd in soil this will result in the Cd being taken up by surrounding plants (ATSDR, 1999; Liu et al., 2003; Sharma et al., 2007). For comparison, three samples of green and red fern leaves were taken at the wet market in the study area. Fern leaves in the wet market are usually brought from outside the study area. Fern leaves were harvested using stainless steel knife and placed in polyethylene plastic bags. The collected fern leaves then cut into small pieces and washed with tap water followed by deionized water. Samples were dried in the oven with temperature 65°C to 70°C to let it dry until constant weight obtained. The dried samples were crushed, grounded by using pastel and mortar, weight using analytical balance and kept in beaker enclosed (Munisamy et al., 2013).

Soil samples were collected using shovel at 0 – 20 cm depth. In total, 10 soil samples were collected at the same spot where the fern leaves were taken. Soil samples collected were transferred to clean plastic sheets or blank white paper and let it air dried for a week. The dried samples were sieved, then crushed, grounded by using pastel and mortar, weight using analytical balance and kept in enclosed beaker (Munisamy et al., 2013).

Digestion method was used to analyse Cd concentration in fern leaves. Dried crushed and grounded samples were accurately weight 0.5g ( $\pm$  0.001g) directly into a flask. The samples then were digested with 15 ml of mixed acids (HNO<sub>3</sub> and HClO<sub>4</sub>, 80:20, v:v) at 120°C on a hot plate. This procedure were carried out in fume chamber (Pongthornpruek et al., 2008). After the complete digestion, samples were filtered through Whatman filter paper No. 42. Every sample solution was made up to 25 ml of final volume with deionized water (Arora et al., 2008). The digested samples were stored in refrigerator to be analysed using Graphite Atomic Absorption Spectrophotometer (GAAS). Soil samples weighted 1.0 g were placed into

polyethylene tube and 0.01M Na<sub>2</sub>EDTA solution (0.01M) were added into the tube and shake using end-over shaker at a speed of 180 rpm at room temperature between 28-33°C for one hour. The suspension of the samples was centrifuged at 5000 rpm for 10 min and the supernatant filtered through 0.45 mm membrane for heavy metal analysis using Graphite Atomic Absorption Spectrophotometer (GAAS) (Qiu et al., 2009).

The transfer factor (TF) is used to determine the concentration of heavy metals from soil to edible parts of fern leaves. The transfer of cadmium from soils to fern leaf was calculated as a ratio of Cd concentration in edible parts of fern leaves divide by Cd concentration in soil (Cao et al., 2010) (Eq.1).

$$TF = \frac{\text{Cd concentration in edible parts of fern leaves}}{\text{Cd concentration in soil}} \quad (\text{Eq.1})$$

Non-carcinogenic and carcinogenic health risk assessment (HRA) was performed to estimate potential health risk to human from Cd exposure via ingestion (Munisamy et al., 2013). The non-carcinogenic health risk due to Cd exposure was evaluated through average daily dose (ADD) and target hazard quotient (THQ) calculation as applied in Eq. 2 and 3. The Lifetime Cancer Risk (LCR) is expressed as in Eq. 4.

$$ADD_{\text{ingest}} = \frac{C_{\text{fern}} \times \text{IngR} \times \text{EF} \times \text{ED} \times \text{CF}}{\text{BW} \times \text{AT}} \quad (\text{Eq.2})$$

$$THQ = \text{ADD} / \text{RfD} \quad (\text{Eq.3})$$

$$\text{Cancer risk} = \text{LCR} = \text{ADD} \times \text{SF} \quad (\text{Eq.4})$$

Table 1 shows the input parameters and data source involved in this study. The ingestion rate (IngR) of vegetables for Malaysian adults by gender, location and ethnic by Izzah& Fatimah (2012) was used in the calculation. The study determined patterns of fruits and vegetable consumption among adults of different ethnics in Selangor, Malaysia. The average body weight of Malaysian adults obtained from Azmi et al., (2009) were used as the reference body weight in the calculation. The study reports the average weight of Malaysian involving 6,775 men and 3,441 women aged 18 – 59 years. The Exposure Factors Handbook (USEPA 2002) was used as a reference for the EF, CF, ED and AT values in ADD calculation. The RfD and SF for Cd from IRIS (2003) and USEPA (2012) was applied in the THQ and cancer risk calculation. If the ratio of THQ is less than 1, the exposed population is unlikely to

experience obvious adverse effects from fern leaf ingestion. If THQ ratio is more than 1, this indicates a chance of non cancer risks effects may occur from the consumption (USEPA, 2012). The acceptable or tolerable LCR for regulatory purposes is in the range of  $1 \times 10^{-6}$  to  $1 \times 10^{-4}$  (USEPA 2002).

### 3. Results

Table 2 shows Cd concentrations in fern leaves and soil sample in this study. Cd were significantly higher in red fern compared to the green fern ( $Z = -2.402$ ,  $p = 0.016$ ). The highest Cd was detected in the red fern sample S1 ( $1.89 \pm 0.05 \mu\text{g}/\text{kg}$ ) and S2 ( $2.26 \pm 0.06 \mu\text{g}/\text{kg}$ ). There was no significant difference of Cd in soil samples in this study ( $t = 0.578$ ,  $p = 0.579$ ). High Cd was detected in sample S3 (red fern) ( $3.32 \pm 0.03 \mu\text{g}/\text{kg}$ ) and S13 (green fern) ( $2.87 \pm 0.005 \mu\text{g}/\text{kg}$ ). Cd concentration in all samples are within the permissible limit of Malaysia Food Regulation (1985) ( $1 \times 10^3 \mu\text{g}/\text{kg}$ ) and Dutch soil reference value ( $8 \times 10^2 \mu\text{g}/\text{kg}$ ).

Cd was significantly higher in the fern roots compared to the shoot ( $Z = -1.984$ ,  $p = 0.047$  for red fern,  $Z = -2.402$ ,  $p = 0.016$  for green fern). The correlation test shows no significant association between Cd concentration in fern leaves and soil samples ( $r = -0.200$ ,  $p = 0.747$  for red fern and  $r = -0.245$ ,  $p = 0.691$  for green fern).

The highest transfer factor (TF) of Cd from soil to the root and shoot was determined in red fern ( $z = 0.200$ ,  $p = 0.047$ ). High TF value was obtained for soil-root in S2 (0.958) and soil-shoot in S1 (0.51) (Table 3). The TF value of fern leaves in this study was higher than parsley ( $0.027 \pm 0.03$ ), onion spring ( $0.022 \pm 0.0065$ ) and lettuce ( $0.12 \pm 0.12$ ) Raaghani et al., (2013).

The THQ of Cd exposure through fern leaves consumption was higher for red fern compared to the green fern but not statistically significant difference ( $z = -0.245$ ,  $p = 0.691$ ) (Table 4). For example the THQ for woman with IR 0.133 kg/day and weight 65 kg was  $1.75 \times 10^{-9}$  for red fern while the THQ for green fern was  $5.30 \times 10^{-10}$ . The THQ value was higher among adult women ( $1.75 \times 10^{-9}$ ) compared to men ( $1.62 \times 10^{-9}$ ).



Figure 1: Study location

Table 1: Parameters used for health risk calculation

Parameter	Symbol	Unit	Value	Source
Target hazard quotient	THQ	-	-	-
Lifetime Cancer Risk	LCR	-	-	-
Average Daily Dose	ADD	µg/kg.day	-	-
Cd concentration in Vegetables	C <sub>fern</sub>	µg/kg	-	-
Ingestion rate	IngR	kg/day	0.133 kg/day: adults 0.193 kg/day: woman 0.153 kg/day: men 0.213 kg/day: rural area 0.129 kg/day: urban 0.136 kg/day: Malays 0.132 kg/day: Chinese 0.126 kg/day: Indian	Izzah& Fatimah (2012)
Exposure frequency	EF	days/year	350	USEPA (2002)
Exposure duration	ED	years	24 years: adults	USEPA (2002)
Cancer slope factor	SF	mg/kg/day	6.3	USEPA (2012)
Conversion factor	CF	Kg/mg	1 x 10 <sup>-6</sup>	USEPA (2002)
Body weight	BW	Kg	60 kg: minimum adult men 70 kg: average adult men 55 kg: minimum adult woman 65 kg: average adult woman	Azmi et al., (2009)
Averaging time	AT	days	8760 non cancer	USEPA (2002)
Oral reference dose	RfD	µg/kg.day	1 µg/kg.day	IRIS (2003)

Table 2: Cd concentration in fern leaves and soil

	Sample	Location	Fern Mean ± SD (dw µg/kg)	Range (dw µg/kg)	Soil Mean ± SD (dw µg/kg)	Range (dw µg/kg)	Shoot Mean ± SD (dw µg/kg)	Range (dw µg/kg)	Root Mean ± SD (dw µg/kg)	Range (dw µg/kg)
Red fern	S1	Oil palm plantation	1.89 ± 0.05	0.26 – 2.26	1.75 ± 0.17	1.53 – 3.32	0.89 ± 0.03	0.17 – 0.89	0.99 ± 0.04	0.15 – 1.62
	S2		2.26 ± 0.06		1.66 ± 0.05		0.65 ± 0.06		1.62 ± 0.02	
	S3		0.80 ± 0.07		3.32 ± 0.03		0.32 ± 0.07		0.47 ± 0.003	
	S4		0.51 ± 0.02		1.53 ± 0.02		0.17 ± 0.003		0.34 ± 0.02	
	S5		0.48 ± 0.03		1.91 ± 0.04		0.33 ± 0.02		0.15 ± 0.02	
	S6	Wet market	0.26 ± 0.003	NA	NA	NA				
	S7		0.38 ± 0.06	NA	NA	NA				
	S8		0.64 ± 0.02	NA	NA	NA				
Green fern	S9	Oil palm plantation	0.48 ± 0.10	0.12 – 0.48	1.78 ± 0.03	1.17 – 2.87	0.27 ± 0.10	0.03 – 0.27	0.21 ± 0.01	0.07 - 0.26
	S10		0.33 ± 0.04		1.26 ± 0.06		0.11 ± 0.02		0.21 ± 0.03	
	S11		0.34 ± 0.02		1.81 ± 0.03		0.08 ± 0.01		0.26 ± 0.02	
	S12		0.13 ± 0.08		1.17 ± 0.03		0.03 ± 0.02		0.09 ± 0.08	
	S13		0.12 ± 0.01		2.87 ± 0.005		0.05 ± 0.01		0.07 ± 0.004	
	S14	Wet market	0.27 ± 0.004	NA	NA	NA				
	S15		0.42 ± 0.004	NA	NA	NA				
	S16		0.21 ± 0.05	NA	NA	NA				

Table 3: The transfer factor (TF) value of Cd in fern leaves in comparison to previous study

Author / year	Sample	Sample	Transfer factor	
			Shoot-soil	Root-soil
This study	Red fern	S1	0.51	0.57
		S2	0.39	0.98
		S3	0.10	0.14
		S4	0.11	0.22
		S5	0.17	0.08
	Green fern	S9	0.15	0.12
		S10	0.09	0.17
		S11	0.04	0.14
		S12	0.03	0.08
		S13	0.02	0.02

Table 4: Health risk to human from fern leaves consumption

Characteristics of population	Vegetables ingestion rate (IR) (kg/person/day) <sup>a</sup>	Target hazard quotient (THQ) for red fern				Target hazard quotient (THQ) for green fern			
		Men		Women		Men		Women	
		70 kg <sup>b</sup>	60 kg	65 kg	55 kg	70 kg	60 kg	65 kg	55 kg
Median consumption	0.133	1.62 x 10 <sup>-9</sup>	1.89 x 10 <sup>-9</sup>	1.75 x 10 <sup>-9</sup>	2.06 x 10 <sup>-9</sup>	4.92 x 10 <sup>-10</sup>	5.74 x 10 <sup>-10</sup>	5.30 x 10 <sup>-10</sup>	6.26 x 10 <sup>-10</sup>
Women	0.193	-	-	2.53 x 10 <sup>-9</sup>	2.99 x 10 <sup>-9</sup>	-	-	7.69 x 10 <sup>-10</sup>	9.09 x 10 <sup>-10</sup>
Men	0.153	1.87 x 10 <sup>-9</sup>	2.18 x 10 <sup>-9</sup>	-	-	5.66 x 10 <sup>-10</sup>	6.60 x 10 <sup>-10</sup>	-	-
Rural areas	0.213	2.60 x 10 <sup>-9</sup>	3.03 x 10 <sup>-9</sup>	2.80 x 10 <sup>-9</sup>	3.31 x 10 <sup>-9</sup>	7.88 x 10 <sup>-10</sup>	9.19 x 10 <sup>-10</sup>	8.48 x 10 <sup>-10</sup>	1.00 x 10 <sup>-9</sup>
Urban area	0.219	2.67 x 10 <sup>-9</sup>	3.12 x 10 <sup>-9</sup>	2.88 x 10 <sup>-9</sup>	3.40 x 10 <sup>-9</sup>	8.10 x 10 <sup>-10</sup>	9.45 x 10 <sup>-10</sup>	8.72 x 10 <sup>-10</sup>	1.03 x 10 <sup>-9</sup>
Malays	0.136	1.66 x 10 <sup>-9</sup>	1.93 x 10 <sup>-9</sup>	1.79 x 10 <sup>-9</sup>	2.11 x 10 <sup>-9</sup>	5.03 x 10 <sup>-10</sup>	5.87 x 10 <sup>-10</sup>	5.42 x 10 <sup>-10</sup>	6.40 x 10 <sup>-10</sup>
Chinese	0.132	1.61 x 10 <sup>-9</sup>	1.88 x 10 <sup>-9</sup>	1.73 x 10 <sup>-9</sup>	2.05 x 10 <sup>-9</sup>	4.88 x 10 <sup>-10</sup>	5.70 x 10 <sup>-10</sup>	5.26 x 10 <sup>-10</sup>	6.21 x 10 <sup>-10</sup>
Indian	0.126	1.54 x 10 <sup>-9</sup>	1.79 x 10 <sup>-9</sup>	1.65 x 10 <sup>-9</sup>	1.96 x 10 <sup>-9</sup>	4.66 x 10 <sup>-10</sup>	5.44 x 10 <sup>-10</sup>	5.02 x 10 <sup>-10</sup>	5.93 x 10 <sup>-10</sup>

Note: <sup>a</sup>The ingestion rate from Izzah et al., (2012) by population characteristic in Malaysia , <sup>b</sup>The body weight from Azmi et al., (2009)

Table 5: The human cancer health risk (LCR) from Cd exposure via fern leaves consumption

Characteristics of population	Vegetables ingestion rate (IR) (kg/person/day) <sup>a</sup>	LCR for red fern				LCR for green fern			
		Men		Women		Men		Women	
		70 kg <sup>b</sup>	60 kg	65 kg	55 kg	70 kg	60 kg	65 kg	55 kg
Median consumption	0.133	1.02 x 10 <sup>-8</sup>	1.19 x 10 <sup>-8</sup>	1.10 x 10 <sup>-8</sup>	1.30 x 10 <sup>-8</sup>	3.10 x 10 <sup>-9</sup>	3.62 x 10 <sup>-9</sup>	3.34 x 10 <sup>-9</sup>	3.94 x 10 <sup>-9</sup>
Women	0.193	1.48 x 10 <sup>-8</sup>	1.73 x 10 <sup>-8</sup>	1.60 x 10 <sup>-8</sup>	1.89 x 10 <sup>-8</sup>	4.50 x 10 <sup>-9</sup>	5.25 x 10 <sup>-9</sup>	4.84 x 10 <sup>-9</sup>	5.72 x 10 <sup>-9</sup>
Men	0.153	1.18 x 10 <sup>-8</sup>	1.37 x 10 <sup>-8</sup>	1.27 x 10 <sup>-8</sup>	1.50 x 10 <sup>-8</sup>	3.57 x 10 <sup>-9</sup>	4.16 x 10 <sup>-9</sup>	3.84 x 10 <sup>-9</sup>	4.54 x 10 <sup>-9</sup>
Rural areas	0.213	1.64 x 10 <sup>-8</sup>	1.91 x 10 <sup>-8</sup>	1.76 x 10 <sup>-8</sup>	2.08 x 10 <sup>-8</sup>	4.96 x 10 <sup>-9</sup>	5.79 x 10 <sup>-9</sup>	5.34 x 10 <sup>-9</sup>	6.32 x 10 <sup>-9</sup>
Urban area	0.219	1.68 x 10 <sup>-8</sup>	1.96 x 10 <sup>-8</sup>	1.81 x 10 <sup>-8</sup>	2.14 x 10 <sup>-8</sup>	5.10 x 10 <sup>-9</sup>	5.95 x 10 <sup>-9</sup>	5.50 x 10 <sup>-9</sup>	6.49 x 10 <sup>-9</sup>
Malays	0.136	1.04 x 10 <sup>-8</sup>	1.22 x 10 <sup>-8</sup>	1.12 x 10 <sup>-8</sup>	1.33 x 10 <sup>-8</sup>	3.17 x 10 <sup>-9</sup>	3.70 x 10 <sup>-9</sup>	3.41 x 10 <sup>-9</sup>	4.03 x 10 <sup>-9</sup>
Chinese	0.132	1.01 x 10 <sup>-8</sup>	1.18 x 10 <sup>-8</sup>	1.09 x 10 <sup>-8</sup>	1.29 x 10 <sup>-8</sup>	3.08 x 10 <sup>-9</sup>	3.59 x 10 <sup>-9</sup>	3.31 x 10 <sup>-9</sup>	3.91 x 10 <sup>-9</sup>
Indian	0.126	9.68 x 10 <sup>-8</sup>	1.13 x 10 <sup>-8</sup>	1.04 x 10 <sup>-8</sup>	1.23 x 10 <sup>-8</sup>	2.94 x 10 <sup>-9</sup>	3.43 x 10 <sup>-9</sup>	3.16 x 10 <sup>-9</sup>	3.74 x 10 <sup>-9</sup>

Note: <sup>a</sup>The ingestion rate from Izzah et al., (2012) by population characteristic in Malaysia , <sup>b</sup>The body weight from Azmi et al., (2009)

The THQ increased with the increased of ingestion rate. The THQ between urban and rural areas showed no significant difference since the vegetable IR is about the same which is 0.213 kg/day in rural area and 0.219 kg/day in urban area. The THQ by races also showed no significant difference as they were consistent with the IR. Concerning the carcinogenic risks, the LCR values for heavy metals in this study were within the limit of  $1 \times 10^{-6}$  to  $1 \times 10^{-4}$  for Cd (Table 5).

## Discussion

Result shows Cd concentration was higher in red fern leaves compared to green fern leaves. High concentration of Cd in red fern leaves was possibly related to the growth locations of fern leaves. Red fern leaves grow at the bushes area near the roadside, while green fern leaves grow at the oil palm plantation. The vehicular exhaust with heavy traffic loads can increase the level of contamination with heavy metals (Oluwole et al., 2013). Transportation activities can contribute to accumulation of heavy metals in roadside soil and plants, which could potentially compromise public health and the environment (Yan et al., 2012).

Vegetables take up metals by absorbing them from contaminated soils, as well as from deposits on different parts of the vegetables exposed to the air from polluted environment (Oluwole et al., 2013). Wide leafy vegetables such as red fern leaves are generally considered to accumulate Cd higher than narrow leaves like green fern leaves. According to Pandey & Pandey, (2012), the concentration of Cd (dry weight-based) are typically highly encountered in plant having wide and rough leaves. Previous study from Pongthornpruek et al., (2008), showed that the red fern leaves from *Blechnaceae* family accumulated higher Ni in the leaves compared to the green fern leaves from *Athyriaceae* family with mean  $\pm$  SD of  $5.403 \pm 1.30$  mg/kg and  $3.742 \pm 0.32$  mg/kg respectively.

About 90% of Cd taken up by plants originated from soil and the other 10% taken from the surrounding (Pandey & Pandey, 2012). The uptake of Cd by plants and vegetables occurs through roots and leaves from water and soil in the form of Cd ion. Additional cadmium is transported to roots by diffusion and mass soil flow. Most of the heavy metals that interact with soils will undergoes several changes in their forms. Part of element gets dissolved in water and become available for plants and vegetables to created health risk or toxicity such as

neurotoxicity, hepatotoxicity, nephrotoxicity, produced via absorption and accumulation process of toxicants (Pandey & Pandey, 2012).

There were many factors that influenced the heavy metal accumulation in soil and plants such as pH, soil texture, soil parent material, type and quantity of soil, organic compounds, soil solution, soil moisture, temperature, and microorganism activities. Soil acidity and soil quality may also exert a profound influence on the availability of elements to plants (Pongthornpruek et al., 2008)

Other possibility for Cd content in soils was from the normal agriculture practices such as fertilizers and pesticide use that might cause enrichment of heavy metals. Application of either liquid or solid soil manure (or their derivatives, compost or sludge) or inorganic fertilisers might become important source of Zn, Cu and Cd. Copper and Zinc present in some manure in the amount which could significantly contribute to their concentration (Nanos & Rodríguez, 2012). Organophosphate pesticide might reach the soil through direct application to the soil surface, incorporation in the top few inches of soil, or during application to crops. Pesticides can also enter ground water resources and surface run-off during rainfall, thereby contributing to the risk of environmental contamination (Akan et al., 2013). The adsorption of pesticide in clay soil and organic matter was weak at the early phase but become strong as the pesticide additives were keep added, thus resulting in lower bioavailability and slower degradation. The additives present in commercial formulations that increase solubility or dispersion of the pesticides are also expected to modify their adsorption. This might explain the lack of relationship between pesticide persistence in soil (Muñoz-Leoz et al., 2013).

Based on the result, Cd level in root was higher than shoot for both red and green fern leaves. Ion uptake by roots correlated with ion concentration in the medium which corresponds with the rate of reaction catalyzed by enzyme in the substrate. If ion concentration in the substrates was high, the barriers would be in the maximum rate until reaching the saturated uptake rate. Phyto-accumulation (phyto-extraction) is the process of accumulating contaminants from the growing media in roots, also called hyper-accumulation. If the heavy metal contents in stems and leaves were higher than in roots, it shows that the hyper-accumulator of the plants was able to optimally translocate the heavy

metals from roots to stem and leaves (Sa'ad et al., 2011).

Concentration of Cd in shoot could be higher than that in roots. This is because the soil is not the only source for contamination that can be transferred to the roots. The plants may absorb the heavy metal from air deposition or other unknown source (Yan et al., 2012) and accumulate the heavy metal at the shoots of plants. Based on study conducted by Pandey & Pandey (2012), the Cd comes through environmental air pollution which directly accumulated, observed and dispersed in aerial part of growing vegetable plant.

There was no association between red and green fern leaves and their respective soils ( $p$  value  $>0.05$ ). Different species of plants had variety abilities to take up and accumulate metals. The heavy metals concentration in fern leaves and soil were not significantly different since the metals were in equilibrium between fern leaves and soil. Elevated levels of heavy metals in soils may lead to uptake by plant but there is not generally found high relationship between concentration in soils and plants. This is because it depends on many different factors such as soil metal bioavailability, plant growth and metal distribution to plant parts. Element mobility and plant availability are very important when assessing the effect of soil contamination on plant metal uptake and related phytotoxic effects. Interaction between metals occurring at the root surface and within the plant can affect uptake as well as translocation and toxicity. Disruption of important physiological function of plants can cause imbalance of nutrients and have adverse effect on the synthesis and functioning of many biologically important compounds such as enzymes, vitamins and hormones of the plants (Fytianos et al., 2001).

In this study, red fern leaves had the highest TF of Cd for shoot-soil and root-soil ( $z = 0.200$ ,  $p = 0.047$ ). High TF for shoot-soil probably because of Cd has a relatively higher mobility in nature and lower retention capacity than other toxic metals (Ok et al., 2011). Whereas high TF for root-soil probably because of the heavy metals may be absorbed by clay minerals, carbonates or hydrous oxides or may be precipitated as metal carbonate, hydroxide and phosphate (Ok et al., 2011). Cd is more easily taken up and transported from roots to the edible parts. The TF values for leafy vegetables were higher than those non leafy vegetables, showing that the leafy vegetables accumulate heavy metals more easily from

the soils than non-leafy vegetables (Wang et al., 2006).

The population for which risk was calculated were determined based on sensitivity to toxic effects. Risk was calculated for average adults aged 18 – 59 years were to highlight the risk to the average person. Results on non – carcinogenic health risk (THQ), shows that the population in this study are within the acceptable risk and unlikely to face adverse health effect due to cadmium exposure from fern leaves. The THQ values calculated also not exceed the Provisional Tolerable Weekly Intake (PTWI) of 2.5  $\mu\text{g}/\text{kg}$  body weight for cadmium. PTWI is an important tool in order to measure the relative cadmium intake through diet and it can be defined as the acceptable level of toxic metal that can be ingested on a weekly basis, as determined by the World Health Organisation (WHO) and the Food and Agriculture Organization (FAO). Weekly basis consumption is used as a step of emphasizing the importance of long term exposure towards a toxic metal rather than daily consumption. The risk mitigation measures could be taken against the rate of toxic compound intake.

There may be many other confounding factors including cadmium bioavailability differences among vegetables, the percentage absorption of dietary cadmium in a population, genetics, lifestyle, diet, and antagonistic or synergistic exposure to other elements, and the effect of aging that may influence the occurrence of cadmium poisoning among the persons. It may also be said that there are protective factors (environmental, dietary or lifestyle) against cadmium toxicity that may interfere with cadmium absorption (Lalor, 2008).

There are several limitations in this study. It is understand that findings in this study possibly biased and not representative, further detailed and comprehensive research should be conducted. Detailed assessment also should include other heavy metals such as lead, mercury and tin that are known to pose threat to human being have to be assessed in order to get an overall picture of human safety towards heavy metals ingestion during vegetable consumption. Heavy metals are ubiquitous in the environment, thus, it has high possibilities to get access into our food chain and pose threat to the consumers.

Exposure of cadmium from multiple sources such as water source, fertilizers and pesticides exposure shall be taken into account. This



comprehensive method of research may predict the health risk of local inhabitants from cadmium exposure via consumption of food crops more comprehensively covering all aspects of exposure.

The health risk calculation also was based from secondary data that cannot be generalized to represent the study area. Future assessment need to consider of taking respondents in the study area as sample and the health risk calculated based on their ingestion rate and specific body weight.

## Conclusion

Overall, results of this study highlights Cd was two times higher in the red fern leaves than the green fern leaves. Cd concentrations was within permissible limit of Malaysia Food Regulation 1985 .Cd in all soil samples were below the specified standard of Dutch reference value. Cd was found to be highly accumulated in the roots and shoots of red fern studied. There was no association between Cd in fern leaves and soil. The highest Cd TF for shoot-soil and root-soil in the red fern leaves indicated that it has higher potential of absorption than the green fern.. The THQ for all the studied population were less than 1 and the LCR values were within the limit of  $1 \times 10^{-6}$  to  $1 \times 10^{-4}$  which highlight the Cd exposure is within an acceptable risk.

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