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Health Risk Assessment of Toxic Metals in Different Types of Lettuce Leafy Vegetables Consumption in Bangkok

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Abstract

The objectives of this study were to: 1) determine the levels of toxic metals, such as arsenic (As), cadmium (Cd), and lead (Pb) in lettuce leafy vegetables sold in Bangkok; and 2) determine any potential health risks associated with exposure to As, Cd, and Pb through consumption of lettuce leafy vegetables. A total of 120 lettuce samples named "red coral (RC)", "green oak (GO)" and "coral lettuce (CL)", which are grown in soil and hydroponic systems, were randomly collected from local fresh supermarkets in Bangkok. Inductively coupled plasma mass spectroscopy (ICP-MS) was used to determine the overall amounts of hazardous metals. The lettuce vegetables which were grown in soil, the total As, Cd, and Pb concentrations in CL, RC, and GO ranged from 0.02 to 0.26 mg/kg, 0.03 to 2.49 mg/kg, and 0.02 to 0.53 mg/kg, respectively. Total As, Cd, and Pb concentrations in CL, RC, and GO for the hydroponically grown lettuce vegetables ranged from 0.01 to 0.09 mg/kg, 0.02 to 0.42 mg/kg, and 0.01 to 0.31 mg/kg, respectively. According to the analysis results, the HI values in adults were significantly higher than ($p<0.05$) those of adolescents and children. Moreover, the HI values of metals through consumption of lettuce vegetables were predominantly dominated by Cd exposure. The HI values of metals were in the following order in the adult population: 3.92 for RC, 2.63 for CL, and 2.04 for GO grown in soil. In contrast, the HI values of hydroponically grown vegetables were in the following order in the adult population: 1.37 for RC, 1.27 for GO, and 0.17 for CL. The present result showed that the HI of metals were higher than 1 (HI>1), indicating an unacceptable level of adverse non-carcinogenic health effects for the population.

Keywords : Leafy lettuce vegetables; Arsenic, Cadmium and Lead; Health risk assessment

Introduction

Carbohydrates, vegetables and fruits, proteins, fats, and dairy are the five basic dietary groups for human well-being and should be consumed on a daily basis since each food offers particular nutritional benefits [1]. Vegetables and fruits are required in greater quantities than other foods such as meat, fish, and beans, as well as milk. In general, vegetables and fruits are beneficial to one's overall health [2]. Among various types of vegetables, leafy vegetables are important dietary components of essential and nutrient-dense foods for human health. Antioxidants, bioactive chemicals (A, C, E), and fibers are abundant in leafy vegetables to reduce the risk of dying prematurely and have a 70% lower risk of heart disease [3]. Consumption of raw and cooked vegetables on a regular basis is good for human growth and development [4] and the prevention of infectious diseases as well as the risk reduction of chronic diseases such as cancer, diabetes, and cardiac disease [5]. Vegetables, on the other hand, can absorb various chemicals and pollutants by root or foliar uptake and accumulate them in different parts of their crops [5]. Moreover, long-term exposure to Hg, As, Pb, and Cd, even at low levels, is widely established to have negative health impacts [6].

Metals in vegetables are being evaluated for potential health concerns in industrialized countries, despite the fact that just a few studies have been conducted in developing countries [7]. Therefore, there is growing interest in the research of toxic metal accumulations and their potential health hazards in humans through the ingestion exposure of leafy vegetable consumption. Particularly, Thailand is one of the developing countries that there have been limited studies on the determinations of toxic metals and the evaluations of their health hazards in foods, especially the leafy vegetables. This study was conducted to 1) determine the levels of toxic metals, such as (As Cd, and Pb) in salad lettuce leafy vegetables sold in Bangkok and 2) determine the potential human health risks of As, Cd, and Pb exposure via salad leafy vegetables consumption. This research is expected to ensure the safety of lettuce vegetable consumption in the Thai population which can be consequently beneficial to prevent public health from the exposure of toxicants through food ingestion.

Methodology

Samples collection

Regarding consumer preferences for leafy vegetable consumption, three different types of lettuce, "red coral (RC)", "green oak (GO)", and "coral lettuce (CL)" which were grown in the soil or hydroponic solution were collected during June-September 2021. RC (n = 40), GO (n = 40), and CL (n = 40) were randomly collected from local markets located in the representative areas of Bangkok. A total of 15 representative districts and 6 fresh supermarkets were chosen for sample collection based on number of registered population and market availability. Approximately 300 g of each vegetable was randomly collected and stored at 4 ^{o}C until sample preparation.

Samples preparation

All samples were washed to remove all soil particles and other residuals using deionized (DI) water at least three times. After that, the inedible parts were discarded. The remaining edible parts were air-dried at room temperature before being dried in a hot air oven at 60 ^{o}C for 48 hours. The moisture content was obtained after weighing the wet weight and dry weight of each sample. Then, each dried sample was ground into a fine powder with a commercial aluminum blender and passed through a 40-mesh (0.420 mm) sieve. Finally, the sample was stored in a desiccator until further analyses.

Samples digestion

Vegetable samples were digested following the US. EPA method. In brief, about 0.50 g of each dried sample was weighed into a 15-mL polyethylene tube. Then, 5 mL of 69% superpure nitric acid (HNO_3) and 5 mL of hydrogen peroxide (H_2O_2) were added into the tube. After that, the tube was capped and left in the hood at room temperature for 48 hours. To obtain a consistent solution, the sample solution was heated in a heating block at 80-90 °C for 15 minutes without boiling before being elevated to 95 °C and boiling for at least 4 hours. The additions of 2.5 mL of HNO_3 and 1.5 mL of H_2O_2 were added into the sample solution when the sample still had brown fume and bubbles. The digestion process was continued until a clear solution was obtained. After the digestion process, the sample was filtered through the Whatman No 40-filter paper. Afterward, 9 mL of deionized water (18.2 MΩ.cm, ELGA PURELAB Maxima) was added into a tube containing the acid digested solution for total metal analyses. This solution was stored at 4 °C until further analysis. The standard reference materials (SRM) of tomato leaf flour (NIST SRM 1573a) as well as blank samples were treated in the same manner as sample digestion to verify the accuracy of the digestion method. The acceptable recovery ranges of As and Cd were obtained (101%).

Instrumental analyses

The total concentrations of As, Cd, and Pb were analyzed by an inductively coupled plasma–mass spectrometry (ICP-MS) (Agilent 7500c, Tokyo, Japan). The SRM of trace elements in water (NIST SRM 1643e) was used to ensure the accuracy of the instrumental analyses. The acceptable recovery ranges of As and Cd were also obtained (97%).

Assessment of human health risk

The potential health risks to humans associated with long-term exposure to these three metals through ingestion of leafy vegetables were calculated using Eqs. 1 to 5.

ADD = (CV x IR x EF x ED) / (BW x AT) (Eq. 1)

Where, ADD is average daily dose (mg/kg·day), CV is concentration of metal in vegetable (mg/kg), IR is ingestion rate (kg·day), EF is exposure frequency (days/year), ED is exposure duration (years), BW is body weight (kg), and AT is average time (days). The input values for the constant variables which were used in this step are summarized in Table 1.

Table 1 Input parameters to characterize exposure values

Variable	Unit	Children (3 to 12.9 years)	Adolescents (13 to 17.9 years)	Adults (18 to 64.9 years)
ED	years	10	5	47
EF	days/year	365	365	365
AT (ED*365)	days	3650	1825	17155
BW [8]	kg	32.29	52.78	70
IR [9]	kg·day	0.00041	0.00086	0.00138

Next, the hazard quotient was estimated to assess the non-carcinogenic risk using Eq. 2.

Hazard quotient (HQ) = ADD / RfD (Eq. 2)

Where, RfD is oral reference dose and ADD is average daily dose (mg/kg. day)

Moreover, a hazard index (HI) following Eq. 3 was used to determine the potential risk to human health when more than one metal was ingested. An exposed population was at an unacceptable risk when HI was greater than or equal to 1.

$HI = HQ_{As} + HQ_{Cd} + HQ_{Pb}$ (Eq. 3)

For the cancer risk assessment, Eqs. 4 to Eq. 5 were used to evaluate the risk of cancer that may develop in human as a result of the ingestion exposure of carcinogen.

LADD = (CV x IR x EF x ED) / (BW x AT) (Eq. 4)

Where, LADD is lifetime average daily dose for cancer risk.

Cancer risk (CR) = SF× LADD (Eq. 5)

Where, SF is a slope factor of particular carcinogen. The values for potential human health risk assessment used in this study are summarized in Table 2.

Statistical analyses

ANOVA was used to determine the significant differences in the concentrations of As, Cd, and Pb accumulated in the different types of vegetables as well as within the same type of vegetable with different cultivation methods. In addition, the significant differences in the potential health risks were determined when more than one metal was ingested in the different groups of the population. A significant level of 0.05 was used in this study.

Results and Discussions

Total concentrations of As, Cd and Pb in different types of lettuce vegetables

Table 3 summarized the total concentrations of As, Cd and Pb contained in the lettuce leafy vegetables.

Table 2 Values of the oral reference dose (RfD) and slope factor (SF) of As, Cd and Pb [10]

Reference values for health effect endpoints	As	Cd	Pb
RfD (mg/kg·day)	3×10^{-4}	1×10^{-3}	3.5×10^{-3}
SF ((mg/kg·day))$^{-1}$	1.5	-	-

Table 3 Concentrations of As, Cd and Pb in lettuce leafy vegetables

Vegetable	Element	Soil cultivation					Hydroponic cultivation				
Red coral		Mini	Max	Mean	Median	SE	Mini	Max	Mean	Median	SE
(n=40)	As	0.01	0.19	0.06	0.04	0.01	0.01	0.10	0.04	0.04	0.00
	Cd	0.03	2.49	0.26	0.25	0.07	0.02	0.42	0.12	0.11	0.01
	Pb	0.02	0.47	0.19	0.13	0.02	0.02	0.29	0.09	0.08	0.01
Green oak	As	0.01	0.08	0.04	0.04	0.00	0.01	0.06	0.03	0.03	0.00
(n=40)	Cd	0.04	0.97	0.23	0.14	0.04	0.05	0.39	0.16	0.15	0.01
	Pb	0.02	0.53	0.15	0.13	0.02	0.01	0.31	0.14	0.15	0.01
Coral lettuce	As	0.02	0.26	0.06	0.05	0.01	0.02	0.09	0.04	0.03	0.00
(n=40)	Cd	0.02	0.98	0.18	0.12	0.03	0.06	0.20	0.11	0.11	0.00
	Pb	0.04	0.37	0.13	0.14	0.01	0.05	0.24	0.12	0.11	0.01
MOPH standard	As	2 mg/kg		Cd	0.2 mg/kg		Pb	0.1 mg/kg			
WHO standard	As	0.1 mg/kg		Cd	0.2 mg/kg		Pb	0.3 mg/kg			

Remark: SE is standard error, MOPH is Ministry of Public Health, WHO is World Health Organization

The total concentrations of As, Cd and Pb in three different types of lettuce leafy vegetables named RC, GO and CL are summarized in Table 3. It was found that As concentrations in RC, GO and CL leafy vegetables cultivated by both methods were lower than the Ministry of Public Health Thailand standard (2 mg/kg) [11]. There were no significant differences ($p>0.05$) in As concentrations in different types of vegetables from the different cultivation methods. In comparison to the World Health Organization's standard of 0.1 mg/kg [12], levels of As in RC and CL were higher than the standard, except GO, which was grown by soil cultivation. When compared to the regulated limits from MOPH and the WHO standard (0.2 mg/kg), it was found that total Cd concentrations in RC, GO, and CL leafy vegetables cultivated in soil were higher than both standards. The statistical analyses indicated significantly higher total Cd concentrations in the RC which was cultivated in the soil than those of other vegetables ($p<0.05$). In addition, Cd levels in RC grown in soil were about 1.5 times greater than GO and CL. All vegetables cultivated in a hydroponic system had higher Cd levels than the standard, except for CL. The total Pb concentrations in leafy vegetables cultivated by both methods were higher than the MOPH standard of 0.1 mg/kg [11]. In addition, when compared to the World Health Organization standard of 0.3 mg/kg [12], levels of Pb in RC, GO, and CL, which were grown by soil cultivation, were higher than the standard. With the exception of CL, the total Pb levels in vegetables that were hydroponically grown were within the WHO limit. Although the Pb contents in vegetables cultivated under two systems were higher than the MOPH standard, there were no significant differences ($p>0.05$) in Pb concentrations in different types of vegetables.

The findings of this study were also compared to those of salad leafy vegetables cultivated in soil reported in previous studies from different countries. Malan et al. [13] reported As concentrations of 1.50 to 4.14 mg/kg in lettuce grown at Philippi horticultural area, which were approximately four times higher than the results of this study. Chunhabundit [14] discovered that leafy vegetables which were obtained from Bangkok's major supermarkets and grocery stores, lettuce, had higher Cd levels than spinach. Farooq et al. [15] discovered that

Pb concentrations in lettuce and spinach grown in Pakistan's industrial districts ranged from 2.25 to 2.41 mg/kg, which was around 1.7 times higher than the Pb concentrations in the current study. Based on the results of total concentrations of all metals of interest found in lettuce vegetables in this present study, the concentration of each metal varied depending on the varieties of leafy vegetables. To begin with, for instance, the maximum As concentration was observed in CL, the maximum Cd concentration was found in RC, whereas the maximum Pb concentration was determined in GO.

This study showed that soil-cultivated vegetables typically contained higher total concentrations of all metals of interest than those of hydroponically grown vegetables. This can be explained by the fact that soil generally contains both essential and non-essential metals, including As, Cd, and Pb. There are several studies reported about metal contamination in agricultural soils. For example, soil samples from the Ayutthaya Province contained contamination levels of As, Cd, and Pb ranging from 0.26 to 1.4, 0.01 to 0.05, and 0.46 to 8.19 mg/kg, respectively, but all the amounts were below the Thai soil quality standard [16]. In addition, it might be the geological properties of soils and parent rocks, as well as anthropogenic activity, such as the application of chemical fertilizer, that can elevate the metal concentrations in soil to higher than natural levels. It was found that the lower pH in soil after nitrogen and phosphate fertilizer applications can cause metal desorption from the soil matrix and result in higher concentrations of As, Cd, and Pb in vegetables [17]. Moreover, agricultural soil pollution by metals is one of the most pressing challenges in China, according to Zhao et al. [3], but it also occurs in Pakistan [18], South Korea [19], India [20] and Serbia [21]). The research described above show that agro-ecosystem contamination is a worldwide problem that has an impact on healthy vegetable production.

Non-carcinogenic health risks from different lettuce leafy vegetables consumptions

1. Overall hazard index (HI) of leafy vegetables consumption

A hazard index (HI) was used to determine the overall potential non-carcinogenic risks to human health when all these three metals (As, Cd, and Pb) were ingested via the leafy vegetable's consumption. The summary of HI values is shown in Table 4. According to the analysis results, the HI values in adults were significantly higher than ($p<0.05$) those of adolescents and children. The present result showed that the HI of the metals was higher than 1 (HI>1), indicating an unacceptable level of adverse non-carcinogenic health effects for the population. The contribution of Cd to the overall HI values was approximately 70%. The HI values were in the following order in the adult population: 3.92 for RC, 2.63 for CL, and 2.04 for GO grown in soil cultivation. In contrast, the HI values of hydroponically grown vegetables were in the following order in the adult population: 1.37 for RC, 1.27 for GO, and 0.17 for CL. The present result showed that the HI of the metals was higher than 1 (HI>1), indicating an unacceptable level of adverse non-carcinogenic health effects for the population.

As a result of HI values greater than acceptable, several health impacts can be developed after long-term consumption of leafy vegetables. For example, the first signs of acute arsenic poisoning symptoms include vomiting, diarrhea, and stomach pain, and long-term exposure can lead to serious health problems like skin cancer [22]. Cadmium has a biological half-life of 10-35 years in humans, with the kidneys being the primary site of accumulation. Diabetes, renal disease, and heart attacks have all been linked to chronic cadmium exposure [23]. High Pb exposure has also been related to impacts on adult reproduction, including decreased sperm count in males and spontaneous abortion in women [24].

2. Carcinogenic health risks from different lettuce leafy vegetables consumption

The cancer risk values in all population groups via consumption of different leafy vegetables grown in different cultivation methods are summarized in Table 5. Since As is the only element which is classified as a Class A cancer according to EPA's Integrated Risk Information System (IRIS) [25], the results of the cancer risk assessment in the study were then evaluated only for the As exposure from the leafy vegetables consumption. The results showed that cancer risk (CR) of As exposure through the soil grown RC vegetable consumption in children, adolescents and adults ranged from 1.67×10^{-7}, 1.07×10^{-7} and 1.21×10^{-6}. In addition, the cancer risk of As exposure in the adult population through lettuce leafy vegetables consumption in this study was within acceptable (cancer risk $\leq 10^{-6}$). Therefore, it can be concluded all groups of the population are safe from cancer risk, though the vegetables are consumed on a daily basis.

Table 4 The values of hazard index (HI) through consumption of different lettuce leafy vegetables

Population	Element	Red coral		Green oak		Coral lettuce	
		Soil	**Hydro**	**Soil**	**Hydro**	**Soil**	**Hydro**
Children	As	0.13	0.08	0.07	0.06	0.17	0.08
	Cd	0.38	0.09	0.18	0.10	0.17	0.06
	Pb	0.03	0.02	0.03	0.02	0.03	0.02
	$HI=HQ_{As}+HQ_{Cd}+HQ_{Pb}$	0.54	0.19	0.28	0.18	0.36	0.16
Adolescents	As	0.08	0.05	0.05	0.04	0.11	0.08
	Cd	0.24	0.06	0.12	0.06	0.11	0.04
	Pb	0.02	0.01	0.02	0.01	0.02	0.01
	$HI=HQ_{As}+HQ_{Cd}+HQ_{Pb}$	0.34	0.12	0.19	0.11	0.23	0.13
Adults	As	0.94	0.61	0.51	0.40	1.22	0.58
	Cd	2.76	0.63	1.31	0.71	1.23	0.45
	Pb	0.22	0.13	0.22	0.16	0.18	0.14
	$HI=HQ_{As}+HQ_{Cd}+HQ_{Pb}$	3.92	1.37	2.04	1.27	2.63	0.17

Table 5 Cancer risk of As exposure though the different lettuce leafy vegetables consumption

Element	Population	Red coral		Green oak		Coral lettuce	
		Soil	**Hydro**	**Soil**	**Hydro**	**Soil**	**Hydro**
As	Children	1.67×10^{-7}	1.13×10^{-7}	1.03×10^{-7}	8.00×10^{-8}	1.65×10^{-7}	1.14×10^{-7}
	Adolescents	1.07×10^{-7}	7.25×10^{-8}	6.62×10^{-8}	5.13×10^{-8}	1.06×10^{-7}	7.31×10^{-8}
	Adults	1.21×10^{-6}	8.20×10^{-7}	7.49×10^{-7}	5.81×10^{-7}	1.20×10^{-6}	8.28×10^{-7}

Conclusion

This study observed the concentrations of As, Cd, and Pb in different types of lettuce vegetables that were sold in Bangkok's fresh markets at the representative areas. The total concentration of each metal was determined, and the health risk assessment was calculated to point out the potential health risk to the population through lettuce vegetable consumption. The concentrations of all metals in soil-grown leafy vegetables were consistently greater than those in hydroponically grown leafy vegetables. For instance, the maximum As concentration was found in soil-grown coral lettuce. Interestingly, red coral had the highest Cd concentration among the different types of vegetables for both cultivations. About 25% of all vegetable samples contained Cd and lettuce consumption was not safe for Cd exposure. The maximum Pb contents were found in green oak, which is grown in soil.

The hazard index (HI) values of metals through consumption of lettuce vegetables were predominantly dominated by Cd exposure. Adults are the group of people who may encounter non-carcinogenic health impacts more often than adolescents and children. According to the non-carcinogenic health risk calculation on the current study results, it is assumed the GO and CL are safe to eat every day rather than RC. For carcinogenic health risk, the cancer risk of As exposure in the population through lettuce consumption in this study was acceptable (cancer risk$\leq 10^{-6}$). Therefore, all groups of the population are safe from cancer risk even though they consume vegetables daily. According to the findings of this study, public awareness of the public health implications of metal contamination should be increased in order to ensure the safety of RC lettuce consumption. Moreover, practical and general recommendations should be introduced to reduce the metal's exposure via lettuce consumption.

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