HEALTH RISK ASSESSMENT OF TOXIC METALS IN COMMONLY CONSUMED SALAD LEAFY VEGETABLES IN BANGKOK

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การประเมินความเสี่ยงต่อสุขภาพของโลหะมีพิษในสลัดผักใบเขียวในเขตพื้นที่กรุงเทพมหานคร

น.ส.ซิน ฮทู ฮยาน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ซิน ฮทู ฮยาน : การประเมินความเสี่ยงต่อสุขภาพของโลหะมีพิษในสลัดผักใบเชียวในเขตพื้นที่กรุงเทพมหานคร. (HEALTH RISK ASSESSMENT OF TOXIC METALS IN COMMONLY CONSUMED SALAD LEAFY VEGETABLES IN BANGKOK) อ.ที่ปรึกษาหลัก : เพ็ญรดี จันทร์ภิวัฒน์

วัตถุประสงค์ของการศึกษานี้ คือ 1) เพื่อตรวจวัดความเข้มข้นของโลหะมีพิษ (สารหนู แคดเมียม และตะกั่ว) ในผักสลัดใบเขียวที่เป็นที่นิยมบริโภคและขายในพื้นที่กรุงเทพมหานคร และ 2) เพื่อประเมินความเสี่ยงต่อสุขภาพของมนุษย์จากการรับสัมผัสสารหนู แคดเมียม และตะกั่ว ผ่านการบริโภคผักสลัดใบเขียว การศึกษาได้สุ่มเก็บตัวอย่างผักสลัดใบเขียวทั้งหมด 120 ตัวอย่างจากตลาดสดในพื้นที่ต่าง ๆ ของกรุงเทพมหานคร และทำการวิเคราะห์ความเข้มข้นทั้งหมดของโลหะมีพิษด้วยเครื่อง Inductively coupled plasma mass spectroscopy (ICP-MS) ผลการศึกษาปริมาณความเข้มข้นทั้งหมดของสารหนู แคดเมียม และตะกั่วในผักกาดหอมแดง (Red Coral) กรีนโอ๊ค (Green Oak) และผักกาดหอม (Coral Lettuce) ที่เพาะปลูกในดินนั้นมีค่าอยู่ระหว่าง 0.0064 ถึง 0.2603 มิลลิกรัม/กิโลกรัม, 0.0216 ถึง 2.4862 มิลลิกรัม/กิโลกรัม และ 0.0151 ถึง 0.5314 มิลลิกรัม/กิโลกรัม ตามลำดับ ในขณะที่ความเข้มข้นทั้งหมดสารหนู แคดเมียม และตะกั่วในผักกาดหอมแดง กรีนโอ๊ค และผักกาดหอมที่เพาะปลูกด้วยระบบไฮโดรปอนิกส์นั้นค่าอยู่ระหว่าง 0.0054 ถึง 0.0990 มิลลิกรัม/กิโลกรัม, 0.0223 ถึง 0.4168 มิลลิกรัม/กิโลกรัม และ 0.0095 ถึง 0.0509 มิลลิกรัม/กิโลกรัม ตามลำดับ ผลการประเมินความเสี่ยงต่อสุขภาพจากการบริโภคผักสลัดใบเขียว พบว่าค่าความเสี่ยงของผลกระทบทางสุขภาพที่มิใช่มะเร็ง (HQ) มีค่าเรียงลำดับจากสูงไปต่ำในกลุ่มประชากรผู้ใหญ่ > วัยรุ่น > เด็ก โดยที่ค่าเฉลี่ยความเสี่ยงของผลกระทบทางสุขภาพที่มิใช่มะเร็งจากการรับสัมผัสสารหนู แคดมียม และตะกั่วในผักสลัดใบเขียวที่เพาะปลูกในดิน มีค่า 0.19 ถึง 0.80, 0.24 ถึง 2.29 และ 0.04 ถึง 0.14 ตามลำดับ ในขณะที่ค่าเฉลี่ยความเสี่ยงของผลกระทบทางสุขภาพที่มิใช่มะเร็งจากการรับสัมผัสสารหนู แคดมียม และตะกั่วในผักสลัดใบเขียวที่เพาะปลูกด้วยระบบไฮโดรปอนิกส์ มีค่า 0.13 ถึง 0.30, 0.11 ถึง 0.38 และ 0.02 ถึง 0.08 ตามลำดับ และพบว่าแคดเมียมในผักกาดหอมแดงนั้นมีค่าความเสี่ยงของผลกระทบทางสุขภาพที่มิใช่มะเร็งเกินเกณฑ์ที่ยอมรับได้ (HQ≥1) ส่วนค่าความเสี่ยงของผลกระทบทางสุขภาพที่มิใช่มะเร็งจากการรับสัมผัสสารหนูและตะกั่วในกรีนโอ๊คและผักกาดหอมนั้นมีค่าต่ำกว่า 1ดังนั้นการบริโภคผักสลัดใบเขียวเป็นประจำในประชากรผู้ใหญ่นั้นอาจก่อให้เกิดผลกระทบต่อสุขภาพโดยเฉพาะในส่วนของโรคที่มิใช่มะเร็งได้

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Zin Htoo Hlyan : HEALTH RISK ASSESSMENT OF TOXIC METALS IN COMMONLY CONSUMED SALAD LEAFY VEGETABLES IN BANGKOK. Advisor: Asst. Prof. Dr. PENRADEE CHANPIWAT, Ph.D.

The objectives of this study were 1) to investigate concentrations of toxic metals including As, Cd, and Pb in the commonly consumed salad leafy vegetables which were sold in Bangkok, and 2) to assess potential human health risks of As, Cd, and Pb exposure via salad leafy vegetables consumption. A total of 120 samples were randomly collected from local fresh markets in Bangkok. The total concentrations of toxic metals were determined by an inductively coupled plasma mass spectroscopy (ICP-MS). Total concentrations of As, Cd and Pb in coral lettuce (CL), red coral (RC) and green oak (GO) for soil cultivation varied from 0.0207 to 0.2603 mg/kg, 0.0280 to 2.4862 mg/kg, and 0.0174 to 0.5314 mg/kg, respectively. For the hydroponic vegetables, total concentrations of As, Cd and Pb ranged from 0.0225 to 0.0943 mg/kg, 0.0223 to 0.4168 mg/kg, and 0.0095 to 0.3091 mg/kg, respectively. According to the health risk assessment, the HQ values of As, Cd, and Pb ranked in the following order: adults > adolescents > children. For soil cultivation, the average HQ values of As, Cd and Pb were 0.19 to 0.80, 0.24 to 2.29, and 0.04 to 0.14. Meanwhile, the average HQ values of As, Cd and Pb exposure from the hydroponically grown salad vegetables were 0.13 to 0.30, 0.11 to 0.38, and 0.02 to 0.08, respectively. According to the HQ values, Cd in RC consumption was higher than the acceptable non-cancer risk of 1 ($HQ \geq 1$). On the other hand, the GO and CL were safe for consumption as the HQ values for As and Pb were lower than 1 ($HQ<1$). As a consequence of the regular lettuce consumption, the adult population may develop non-cancer health risks (e.g., diabetes, kidney disease, and heart attack).

Field of Study:	Hazardous Substance and Environmental Management	Student's Signature ....
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LIST OF ABBREVIATIONS

As	Arsenic
Cd	Cadmium
Pb	Lead
WHO	World Health Organization
FAO	Food and Agriculture Organization
MOPH	Ministry of Public Health
US EPA	United States, Environmental Protection Agency
ATSDR	Agency for Toxic Substances and Disease Registry
IP-HSM	International Program on Hazardous Substance and Environmental Management
ERIC	Environmental Research Institute
ER	Exposure rate
CV	Concentration in vegetables
IR	Ingestion rate
EF	Exposure frequency
ED	Exposure duration
BW	Body weight
AT	Average time
HQ	Hazard quotient
HI	Hazard index
RfD	Oral reference dose
SF	Slope factor
LADD	Lifetime average daily dose
˚C	Degree celsius
mg	Milligram
kg	Kilogram
mg/kg·day	Milligram kilogram per day
Days/years	Days per years
CR	Cancer risks

μg/L	Micrograms per liter
HNO_3	Nitric acid
H_2O_2	Hydrogen peroxide
H_2O	Water
SRM	Standard reference material
ICP-MS	Inductively coupled plasma mass spectrometry
TNFI	Thailand National Food Institute's
IRIS	Integrated Risk Information System
Min	Minimum
Max	Maximum
SE	Standard error

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CHAPTER I
INTRODUCTION

1.1 Background of the study

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 (Gupta et al., 2021). 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. Basically, vegetables normally contain more protein and fiber than those of fruits. Therefore, vegetables are essential components of a healthy diet as they contain various vitamins, minerals, and even carbohydrates (Aysha et al., 2017). Appropriate consumption of vegetables on a regular basis may consequently help to prevent severe disease including cardiovascular disease and certain cancer.

Among various types of vegetables, leafy vegetables are important dietary components as well as essential and nutrient-dense foods for human health. Antioxidant vitamins and bioactive chemicals (A, C, E) as well as minerals like calcium (Ca), magnesium (Mg), copper (Cu), manganese (Mn), zinc (Zn), potassium (K), carbohydrates, vitamins, and fibers are abundant in leafy vegetables (Zhou et al., 2016). Consumption of raw and cooked vegetables on a regular basis is good for human growth and development (Ikem & Egiebor, 2005) and the prevention of infectious diseases as well as the risk reduction of chronic diseases such as cancer, diabetes and cardiac disease (Babandi et al., 2020). According to the World Health Organization (WHO)'s fruits and vegetables campaign launched in 2003, an individual person should consume more than 400 grams of fruits and vegetables per day, or approximately 150 kilograms per year. However, it was found that in recent years, people all over the world have consumed more fresh vegetables than ever before in order to preserve nutritious vitals, a balanced body, and live healthy lives. Therefore, it can be implied that vegetable consumption is a significant way of obtaining critical nutrients.

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 (Briffa et al., 2020). The accumulation of pollutants, particularly toxic metals, in vegetables can be caused by both natural and anthropogenic sources. The environmental media causing the accumulation of toxic metals in plants are including soil, irrigation water, fertilizer as well as other cultivation environments. For example, a study of (Gupta et al., 2021), has found the accumulations of poison metals in vegetables and fruits as a result of fertilization, irrigation, and air. Another study reported by Luo et al. (2011) found significant Cd levels of 0.79 mg/kg in broccoli and Pb levels of 0.38 mg/kg in lettuce which were cultivated in the contaminated areas around an e-waste processing site in China. Gupta et al. (2008) specifically reported the accumulations of 57.63 mg/kg of Pb and 17.79 mg/kg of Cd in radish as a result of fertilization and irrigation of wastewater in the suburban area in Titagarh, India. When considering organic and conventional cultivation practices, Li et al. (2018) found comparable concentrations of As, Pb, and Cd in five leafy vegetables studied (7.86 mg/kg of As, 9.17 mg/kg of Pb, and 12.1 mg/kg of Cd in organic vegetables and 7.29 mg/kg of As, 15.3 mg/kg of Pb, and 17.9 mg/kg of Cd in conventional vegetables). These examples clearly shown that toxic metals deposited in the environment especially in the vegetable cultivation media can cause the elevated accumulation of metals in the harvesting vegetables. This can consequently cause a public health concern over the food safety as well as the negative impacts to the consumers and other flora and animals. Therefore, it can be concluded that leafy vegetables can not only be an important source of minerals and vitamins for health of human well-being, but they can also be a source of hazardous metal exposure in humans.

The increasing levels of hazard elements in vegetables which could have an influence on human health have been recorded in modern years. For example, Hughes et al. (2011) found that leafy vegetables normally have higher Pb and Cd concentrations than that of the root vegetables, with lettuce having the highest concentration. When humans ingest vegetables with high levels of heavy metals, they can cause a variety of clinical and physiological issues (Xu et al., 2015). Metals such as Cd, chromium (Cr), Pb and mercury (Hg) are examples of metals with density higher than 5 g/cm^3 that can cause toxic effects to humans. Since metals are persistent, non-biodegradable, biomagnified through the food chain and some are highly toxic to human health, their accumulations in foods are one of the public health concerns during these recent years. Metals build up in the human's key organs, such as bones, liver and kidneys are linked to a variation of major health problems (Duruibe et al., 2007). Vegetables with trace levels of some essential metals are necessary for growing of animals and human. However, their concentrations should be kept within the regulated levels. On the other hand, high levels of Pb and Cd in vegetables and fruits have been linked to a growth risk of stomach and intestine cancer (Türkdoğan et al., 2003). Moreover, long-term exposure to Hg, As, Pb and Cd, even at low levels, is widely established to have negative health impacts (Llobet et al., 2003). Lead (Pb) has shown to have a negative impact on children's growth of brain, excessive Pb in the blood, high blood pressure, damage of kidney function, and heart attack disease (Navas-Acien et al., 2007). Chronic Cd exposure can cause acute liver and lung toxicity, deterioration in kidney function, hearing loss, as well as immune system impairment (Ikem & Egiebor, 2005). Even though As is a metalloid element, it may also cause breast, spleen and skin cancer (Hartley & Lepp, 2008). 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.

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 (Baghaie & Fereydoni, 2019). 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. According to the Thailand's recent food act, there has been a reform in the strategy as well as a growing interest especially in the aspect of food safety to various organizations including governments, public health related departments, and researchers. The recent act mainly focuses on the determinations of toxic metal and bioactive compounds in various types of foods. Therefore, this research is expected to ensure the safety of vegetable consumption in the Thai population which can be consequently beneficial to prevent public health from the exposure of toxicants through food ingestion.

1.2 Objectives of the study

A goal of this present study was to ensure the safety of commonly consumed salad leafy vegetables and to protect human health impacts from the exposure of toxic metals via the consumption of salad leafy vegetables. The sub-objectives of this study were;

1. To investigate the concentrations of toxic metals including As, Cd, and Pb in the commonly consumed salad leafy vegetables which were sold in Bangkok.
2. To assess potential human health risks of As, Cd, and Pb exposure via salad leafy vegetables consumption.

1.3 Research hypotheses

1. Significant higher concentrations of As, Cd, and Pb will be found in the soil grown vegetables comparing to those in the hydroponically grown vegetables.
2. Concentrations of As, Cd, and Pb exceeding the Ministry of Public Health (MOPH) allowable limits will be found in red coral and green oak.
3. The non-carcinogenic health effects of multi-metal exposure from leafy vegetables consumption will be greater than the acceptable health risks.

1.4 Research expected outcomes

1. The assurance of food safety especially on salad vegetables.
2. The potential human health risks of toxic metals exposure from the consumption of salad leafy vegetables.

1.5 Scope of the study

This present study consists of two main parts including i) the determinations of toxic metal concentrations accumulated in the soil grown vegetables and hydroponically grown vegetables, and ii) the assessment of potential human health risks from toxic metal exposure through leafy vegetables consumption.

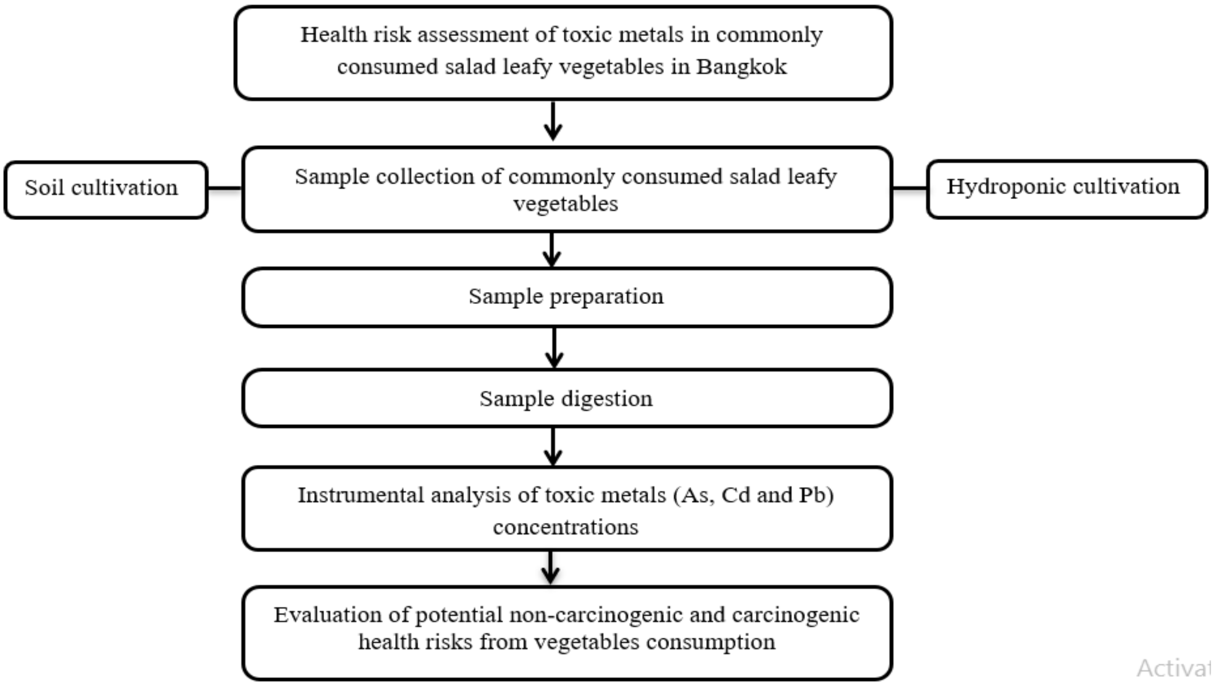

Figure 1 A conceptual framework of the study.

For the first part of the study, types of commonly consumed salad leafy vegetables were selected based on the consumer's preferences. A total of 120 samples were randomly collected from local fresh markets located in the representative areas of Bangkok. Following sample collection, samples were thoroughly washed, dried, ground, and digested for the analyses of total As, Cd and Pb concentrations by an inductively coupled plasma mass spectroscopy (ICP-MS).

Regarding the results of instrumental analyses obtained from the first part of the study, the potential adverse health effects of daily leafy vegetables consumption in the different groups of population in Bangkok were evaluated.

CHAPTER II

LITERATURE REVIEW

2.1 Metal distribution in the environment and uptake by soil and plants

Heavy metals have evolved into significant toxicants due to their tendency to concentrate and bioaccumulate in the food chain and their increasing capacity to dissolve in water when discharged directly or indirectly (Pamonpol & Tokhun, 2019). Even though the term "heavy metal" is widely debated, science defines heavy metals as any kind of metal that demonstrates toxicity regardless of its atomic mass or density, including lead (Pb), mercury (Hg) arsenic (As) and cadmium (Cd) (Raychaudhuri et al., 2021). Reactive oxygen species (ROS) are produced when essential and non-essential heavy metals are present in excess amounts in the soil. ROS has unpaired electrons, which harms plants through oxidative stress, biomass accumulation, chlorosis, inhibition of growth and photosynthesis, and eventually leading to death (Singh et al., 2016) . Macronutrients, or necessary elements like Cu, Fe, and Mn, are needed by plants for development and metabolism. Even in trace concentrations, plants do not need non-essential elements like Cd, Pb, and Hg (Mourato et al., 2015). Understanding the origins of the point or nonpoint sources of pollutants is crucial before we discuss the many types of pollutants. The primary source of nutrition for growing crops is the soil, which can be seriously harmed by heavy metals from point sources like thermal power plants and coal mines, chemical industries like gold mines, smelting, electroplating, textile manufacturing, and e-waste processing, as well as non-point sources like agricultural runoff and soil/sediment erosion (Rai et al., 2019).

Although some metals are necessary for the growth and development of plants, others are not. It is noteworthy to notice that many of these components have been important to daily life from ancient times and still are (Yang et al., 2010). The continual exposure of plants to heavy metal-induced toxicity has a detrimental effect on food crops such as rice, leafy vegetables, wheat, and maize (Raychaudhuri et al., 2021). Since many heavy metals are thought to be necessary for plant growth, heavy metal toxicity in plants varies with plant species, specific metal, concentration, chemical form, soil composition, and pH (Nagajyoti et al., 2010). All year-round vegetables are vulnerable to pest infestations, including aphids and whiteflies, due to the high relative humidity. Tropical regions are more prone to pest problems when growing vegetables because of their higher relative humidity and warmer temperatures (Norsuwan et al., 2021). Chemical N:P: K fertilizers (12:12:12) were applied at rates of 8.34 g/m^2 before planting and 1.1 g/m^2 at 15-25 days following transplanting to lettuce production fields. According to Norsuwan et al. (2021) the yield rates (kg/m^2) for leaf lettuce varied depending on the growth season, ranging from 0-39 to 0.69 during the dry season to 0.42 to 0.74 during the wet season to 0.96 to 1.70 during the winter. Nitrogen (N) runoff from fertilizers used on vegetables is turning into a significant environmental issue. Agriculture was the source of 57 percent of the nitrogen (N) that entered waterways. According to Min and Shi (2018) the research, 80% of N discharge from farmland used for intensive vegetable farming occurs through leaching, 17% through runoff, 2 percent through N_2O emission, and 1 percent through volatilization.

2.2 Metal contamination in the crops

2.2.1 Metals contamination in the soil grown crops

In general, soil contains both essential and non-essential metals in different amounts. The elevated concentrations of metals in soil to the levels higher than that of the natural concentrations can be caused by geological features of soils and parent rocks as well as the anthropogenic activities. Elevated concentrations of metals in agricultural soils can be found after the application of inorganic fertilizers or liquid and soil manure (compost or sludge) into both uncovered and greenhouse vegetable field (Atafar et al., 2010). In addition, dumping of industrial and urban wastes and some industrial operations such as mining have been resulted to cause high metal concentrations in agricultural soils and plants, particularly Cd and Pb (Song et al., 2021). As a result of crop cultivation, the soil grown crops can accumulate those metals in their products. It was concluded that the key causes of metal contamination in agricultural farm vegetables are high fertilizer application and sewage irrigation. The inclusion of large amounts of Cd in some fertilizers is particularly concern for harmful and capacity of this metal to store in soils and bioaccumulation in animals and plants. Superphosphate and lime fertilizer also include toxic metals like Cd, which can build up in the soil, absorbed by plants, and passed on to other organisms through the food chain (Huang & Jin, 2008). Applications of nitrogen and phosphate fertilizer on agricultural fields can result in higher levels of Cd, As, Cr, and Pb in the soil and notably reduce in soil pH which causes metal desorption from the soil matrix (Wångstrand et al., 2007).

Consequently, many studies have reported elevated concentrations of metals in vegetables. For examples, Sharma et al. (2007) reported Cd, Ni, and Pb contents exceeding the allowed limits of the Indian standard in an edible part of *Beta vulgaris* as a result of wastewater irrigation. This study has caused public health concern as *Beta vulgaris* (palak) is a highly nutrient-dense leafy vegetable that is widely produced and consumed in urban India, particularly among the poor. Another study in China Zhou et al. (2016) reported As, Cd, and Pb contamination in vegetables grown on metals polluted soil with the concentrations of 0.207-0.460 mg/kg, 0.540-1.162 mg/kg, and 0.075-0.739 mg/kg, respectively. In addition to the determination of metal concentration found in the vegetables, Zhou et al. (2016) found out that the concentrations of those three metals (As, Cd and Pb) were normally higher in the leafy vegetables comparing to the other types of vegetables.

2.2.2 Metals contamination in the hydroponically grown crops

With the fastest increasing of manufacturing and urbanization, agricultural land is decreasing, and most of the field is reducing fertility while becoming greater contaminated, particularly in peri-urban zones that produce decaying vegetables (Liu et al., 2016). Therefore, hydroponic farming is currently gaining popularity around the world due to its effective resource management and high-quality food production (Sharma et al., 2018). This cultivation method only rely on a nutrient-rich solution with water base (Zimmermann & Fischer, 2020). Since this cultivation method has resulted in 30% of faster growth rate and higher yield than that of the soil cultivation, the hydroponic cultivation technique has become the most popular vegetable cultivation practice (Sharma et al., 2018). For Thailand, in particular, this hydroponic or a soilless cultivation has grown in popularity since 1997, following the country's economic crisis (Wattanapreechanon & Sukprasert, 2012).

Despite the fact that the hydroponic cultivation has more advantages than soil cultivation, metals can still be found in the hydroponically grown vegetables because pesticides are still used to protect against certain pests and diseases (Resh, 2012) as waterborne infections can easily transfer from one plant to another in a hydroponics system (Antisari et al., 2015). For the vegetables grown in the hydroponic system, it is interesting to note that more metals tend to be accumulated in the young plants more than that of the mature plants, especially leafy vegetables, (Hadayat et al., 2018; Zimmermann & Fischer, 2020). The contaminations of metals in the hydroponically grown vegetables were also reported by many researchers. For example, (Liu et al., 2016) found the level of Pb exceeding the Chinese standards in four Chinese flowering cabbage samples and one Italian lettuce sample, while higher As level than the standard was found in two Italian lettuce samples. Hu et al. (2015) have also reported As, Pb, Cr, Cd and Hg contamination in vegetables which were collected from three hydroponic gardens in Hangzhou, China.

2.3 Human health effects of toxic metals

Adverse human health effects of three toxic metals of interests in this present study are summarized as follow.

2.3.1 Arsenic (As)

Arsenic is a naturally existing substance that can be found in food, water, soil, and air. It is a highly toxic substance which is posing to threat both humans and environment. It can occur in a variety of oxidation states and forms (Tchounwou et al., 2012). Humans can come into contact with As through natural sources, industrial sources, or unintended sources. A number of epidemiological studies have discovered a strong link between growing risk of cancer and other systemic health consequences resulting from long-term exposure of As (Walker et al., 2012). Vomiting, stomach pain, and diarrhea are the first signs of acute arsenic poisoning. Meanwhile, long term exposure to inorganic As which is mostly found in drinking water and food can cause several health problems including skin lesions and skin cancer (Argos et al., 2010).

2.3.2 Cadmium (Cd)

According to the Agency for Toxic Substances and Disease Registry (ATSDR), Cd is the seventh most hazard metal. Humans are most often exposed to Cd by inhalation and ingestion and can develop acute and chronic intoxications as a result. Cadmium accumulates mostly in the kidneys and has a biological half-life of 10–35 years in humans (WHO, 2019). Chronic Cd exposure has been found to be related to osteoporosis, kidney disease, diabetes, cancer and heart attack. There is an adequate evidence for their carcinogenic in humans because of long-term and high level occupational exposure to Cd (e.g., through cadmium fume) (Ashizawa et al., 2012).

2.3.3 Lead (Pb)

Lead is the most common and highly toxic metals. Its widespread use has caused severe contamination especially in the atmosphere and caused health issues in many parts of the world. In a dry atmosphere, Pb is a bright silvery metal that is slightly bluish (Tchounwou et al., 2012). Lead has a wide range of commercial, agricultural, and domestic uses. Lead-containing soil, pesticides, or Zn fertilizers may be taken up by roots, plants, or accumulated on leafy plants. Flora et al. (2006) found that plants which are grown in fields or home gardens nearby central roads can be contaminated by Pb pollution from cars or industry. Lead poisoning is normally caused by the inhalation of dust particles as well as the ingestion of Pb contaminated food, water, and paints. In both adults and infants, the nervous system is the most sensitive to Pb poisoning. Generally, kidney absorbs the most Pb in the human body, followed by the other organs including the heart and brain; however, Pb in the skeleton accounts for the majority of the body's Pb (World Health Organization, 2019). There have been several studies that show the harmful effects of Pb on children and adults in which high blood Pb level usually lower IQ, hearing acuity, speech and cause language impairments, growth delaying, and a symptom of anti-social in adolescents. High Pb exposure has been linked to reproductive effects in adults, such as reduced sperm count in men and spontaneous abortions in women (Atsdr, 2007).

Table 1 summarizes the health effects, routes of exposure as well as sources of exposure of the three toxic metals of interests in this study.

Table 1 Health effects, routes of exposure as well as sources of exposure of As, Cd and Pb

Metal	Carcinogen classification	Health effects	Route of exposure	Source of exposure
As	Class A	Hyperpigmentation, keratosis and possible vascular complications	- Ingestion of food and water - Inhalation of background air	- Geological: rocks, soil, water (ground water) - Industry - Burning fossil fuels, - Manufacturing of pesticides

				- Wood preservatives
Cd	Class B	Kidney (significant Proteinuria), gastrointestinal symptoms, nausea, vomiting, abdominal pain, skeletal and respiratory systems	- Ingestion of contaminated food - Inhalation of tobacco smoke	- Volcanic and weathering - Mining, - Tobacco smoking - Smelting, - Fossil fuel combustion - Incineration of batteries and plastics
Pb	Class B	Neurotoxicity, developmental and hearing-impaired delays, lower IQ and male reproductive impairment	Ingestion of contaminated water and food - Inhalation of Pb particles	- Industry - Smelting - Manufacturing of recycling activities - Painting - Jewelry, - Toys - Some cosmetics and traditional medicines

2.4 Regulated concentrations of toxic metals in vegetables

The allowable concentrations of As, Cd, and Pb in vegetables regulated by the World Health Organization (WHO) and the Ministry of Public Health (MOPH) of Thailand are summarized in Table 2, respectively.

Table 2 Allowable concentrations of As, Cd, and Pb in leafy vegetables

Organization	Type of food	As	Cd	Pb
FAO/WHO Food Standards Joint Program	Leafy vegetables and oils	0.1 mg/kg	0.2 mg/kg	0.3 mg/kg
Ministry of Public Health of Thailand (No.414)	Leafy vegetables and Brassica	2 mg/kg	0.2 mg/kg	0.1 mg/kg

It might be assumed that the WHO standard is representative of worldwide international standards. The MOPH standard is represented by the National Thailand standard. Both organizations have different standards for As and Pb. For example, it might be several factors based on assumptions and previous literature review. Vegetable consumption rates in Thailand are lower, but they may be higher on an international scale. The WHO standard is higher than the MOPH standard for lead (Pb).

2.5 Contaminations of As, Cd and Pb in leafy vegetables and their potential health risks

Since metals are non-biodegradable, have long biological half live and can be accumulated in living organisms such as plants and animals (John, 2002). Thus, cultivated vegetables can absorb and accumulate metals in their whole structure (both inedible and edible parts) to the levels that may exceed the recommended limits (Tasrina et al., 2015). Metals concentrations in vegetables (leafy, root, and fruit) have been found to be extremely high in recent years. Therefore, consumption of the contaminated vegetables may have negative health consequences to consumers. The contaminations of As, Cd and Pb over the standard limits particularly the WHO standards have been reported in various vegetables in Huludao City, China with the maximum Cd and Pb concentrations of 0.003–0.195 mg/kg and 0.003–0.624 mg/kg (fresh weight), respectively (Zhou et al., 2016). According to the study of (Zhou et al., 2016), leafy vegetables have shown higher Cd uptake and accumulation than that of the non-leafy vegetables. In the case of Thailand, Chinnawat (2012) has reported Pb and Cd contaminations in basil, coriander plants, ginger, lemon grass, parsley, turmeric, and onion. In addition, Ammara and Ratchai (2016) found the levels of hazardous metals higher than the Thailand National Food Institute's recommendation (TNFI) in two of the vegetables sampled (lettuce and gotu gola).

Table 3 summarizes the concentrations of As, Cd and Pb found in the commonly consumed leafy vegetables in Thailand and other countries.

Table 3 Concentrations of As, Cd and Pb in most consumed leafy vegetables in Thailand and other countries

Study area	**Standard of metals for leafy vegetables (mg/kg)**			**Concentrations found in leafy vegetables (mg/kg)**			**Reference**
	As	**Cd**	**Pb**	**As**	**Cd**	**Pb**	
Chinese cabbage							
Nine provinces in lower north of Thailand	2	0.2	0.1		0.38 - 2.64	1.25 - 5.19	(Wachirawongsakorn, 2016)
Nakhon Pathom Province, Thailand					0.03		(Choprathumma et al., 2021)

Hunan Province, China				0.02 - 0.07	0.03 - 0.42	0.13 - 0.75	(Zhou et al., 2016)
Chinese kale							
Nine provinces in lower north of Thailand	2	0.2	0.3		0.05 - 2.23	1.20 - 4.90	(Wachirawongsakorn, 2016)
Lettuce							
Nine provinces in lower north of Thailand	2	0.2	0.1		0.91 - 2.21	1.05 - 6.52	(Wachirawongsakorn, 2016)
Nakhon Pathom Province						0.05	(Choprathumma et al., 2021)
Hunan Province, China				0.09 - 0.66	0.21 - 0.46	0.54 - 1.16	(Zhou et al., 2016)
São Paulo, Brazil					0.07	0.44	(Guerra et al., 2012a)
Spinach							
Hunan Province, China	2	0.2	0.1	0.06 - 0.31	0.06 - 0.51	0.19 - 0.97	(Zhou et al., 2016)

CHAPTER Ⅲ
METHODOLOGY

3.1 Sample collection

Regarding consumer preferences for leafy vegetable consumption, three leafy vegetable varieties namely, "red coral (RC)", "green oak (GO)", and “coral lettuce (CL)” were collected during June-September 2021. A total of 120 samples cultivated with soil and hydroponics such as 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 population and market availability in the Bangkok area as shown in Figure 2. Approximately 300 g (wet weight) of each vegetable was randomly collected and stored at 4 ^{o}C until sample preparation.

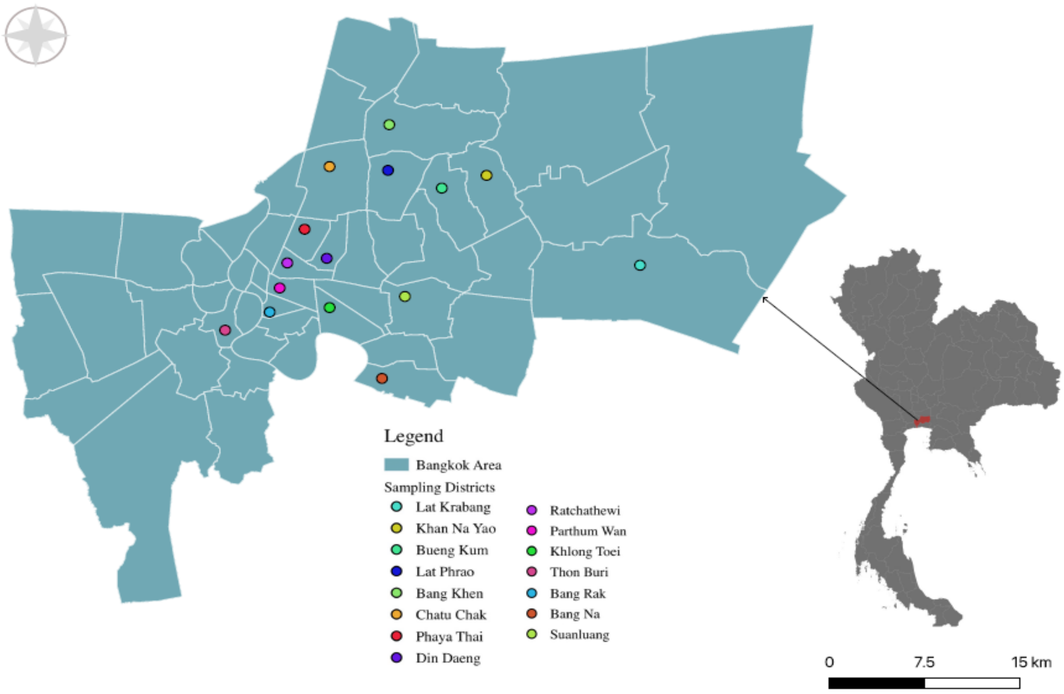

Figure 2 A maps showing the representative districts for sample collections in the study

Figure 3 Pictures showing the type of leafy vegetables included in this study (a) red coral, (b) green oak and (c) coral lettuce

3.2 Sample preparation

After delivery to the laboratory, all samples were washed to remove all soil particles and other residuals by the deionized (DI) water for at least three times (Choprathumma et al., 2021). After that, the inedible parts were discarded. The remaining edible parts were air-dried at room temperature before drying in a hot air oven at 60^{o}C for 48 hours (Choprathumma et al., 2021). The moisture content was obtained after weighing wet weight and dry weight of each sample. Then, each dried sample were grounded into a fine powder with a commercial aluminum blender and passed through a 40-mesh (0.420 mm) sieve. Finally, the sample was kept in a centrifuge tube and placed in desiccator until further acid digestion.

3.3 Sample digestion

Approximately 0.50xx grams of each dried sample were weighed into a digestion polypropylene tube. After that, 5 mL of 69% v/v of ultrapure nitric acid

(HNO_3) and 5 mL of hydrogen peroxide (H_2O_2) were added into the sample tube. Then, the sample was digested following the US EPA 3050B method of sample digestion. 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. Afterward, the additions of 2.5 mL of HNO_3 and 1.5 mL of H_2O_2 were added into the sample solution. The digestion process was continued until a clear solution is obtained. Duplications of each sample digestion were conducted in this study. The standard reference materials (SRM) of tomato leaves 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. In addition, the SRM of trace elements in water (NIST SRM 1643e) was used to ensure the accuracy of the instrumental analyses. After digestion process, the sample was filtered through (No. 40 Whatman filter paper). Finally, 9 mL of deionized water (18 .2 MΩ.cm, ELGA PURELAB Maxima) was added into a tube containing acid digested solution. According to the NIST SRM released list, there is no standard reference material for lettuce. So, it has been using tomato leaf flour to test the digestion method's accuracy.

3.4 Instrumental analyses

The total concentrations of As, Cd, and Pb in the final solution was determined by the ICP-MS (Agilent 7500c, Tokyo, Japan).

3.5 Health risk assessment of vegetables consumption

The average daily dose (ADD) of As, Cd and Pb exposure, hazard quotient (HQ), hazard index (HI), and carcinogenic risk (CR) were calculated to determine the possible human health risks associated with the long-term exposure of these three metals through the ingestion of leafy vegetables following Eq. 1 to Eq. 5 (US. EPA, 1977). Potential health risks of three population groups (children, adolescents, and adults) were evaluated in this study. The input values for the constant variables which were used in this step are summarized in Table 4.

$$ADD = (CV \times IR \times EF \times ED) / (BW \times AT) \qquad \text{Eq. 1}$$

Where,

ADD = Average daily dose (mg/kg·day)

CV = Concentration of metal in vegetable (mg/kg)

IR = Ingestion rate (kg/day)

EF = Exposure frequency (days/year)

ED = Exposure duration (years)

BW = Body weight (kg)

AT = Average time (days)

The data on the IR was obtained from the national report on the food consumption data (MOAC, 2009) of Thailand while, the data on body weight was obtained from FAO report (FAO, 2012).

Table 4 Input parameters to characterize exposure values

Variable	Unit	Children (3-12.9 years)	Adolescents (13-17.9 years)	Adults (18-64.9 years)
ED	years	10	5	47
EF	days/year	365	365	365
AT (ED·365)	days	3650	1825	17155
BW (FAO, 2012)	kg	32.29	52.78	70.36
IR (MOAC, 2009)	mg/kg·day	0.00041	0.00086	0.00138

Following the ADD calculations, the HQ which is an indication of the non-cancer endpoint risks, were evaluated as shown in Eq.2. Theoretically, HQ is a ratio of ADD to an oral reference dose (RfD) with potential health effects to be occurred in human. The RfD of all metals of interests in this study are summarized in Table 5. An exposed population is at risk if the HQ is greater than or equal to one.

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

Where,

ADD = Average daily dose (mg/kg·day)

RfD = Oral reference dose of metal of interest

A hazard index (HI) was used to determine the potential risk to human health when more than one metal was ingested (Eq. 3). An exposed population was at unacceptable risk when HI is greater than or equal to one.

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

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

$$LADD = (C \times IR \times EF \times ED) / (BW \times AT) \qquad \text{Eq. 4}$$

Where,

LADD = Lifetime average daily dose (mg/kg·day)

CV = Concentration of metal in vegetable (mg/kg)

IR = Ingestion rate (kg/meal)

EF = Exposure frequency (days/year)

BW = Body weight (kg)

AT = Average time (days)

$$\text{Cancer risk (CR)} = SF \times LADD \qquad \text{Eq. 5}$$

Where,

SF = Slope factor (mg/kg·day)

LADD = Lifetime average daily dose (mg/kg·day)

It should be noted that the average time (AT) for the cancer risk assessment was normally equal to the multiplication of 70 years (human lifespan) to the total of 365 days per year (70 years x 365 days/year). As a result of cancer risk calculation, CR of 1×10^{-4} to 1×10^{-6} is the acceptable safe range of cancer risk for people.

Table 5 Values of the oral reference dose (RfD) and slope factor (SF) of As, Cd and Pb

Reference value 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	-	-

3.6 Statistical analyses

The IBM SPSS statistical package, Version 22, was used to conduct the statistical analysis. 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 in the different groups of population were determined. A significant level of 0.05 was used in this study.

CHAPTER Ⅳ
RESULTS AND DISCUSSION

4.1 Results of quality control and quality assurance

The standard reference material (SRM 1537a) and trace elements in water (SRM 1643f) were used to ensure the accuracy of the methods of acid digestion and instrumental analysis in this study. Table 6 shows the certainty of the acid digestion procedure. While the precision of the analytical method was summarized in Table 7. According to the recovery results shown in both tables, all metal interests for both methods were within ±15% of the certified values, except for Pb.

Table 6 Recovery rate of metal of interests obtained from the acid digestion samples

Element	Certified value (mg/kg)	Experiment value (mg/kg)	Recovery rate (%)
As	0.1126	0.1039	92.27
Cd	1.517	1.339	88.26

Table 7 Recovery rate of instrumental analysis

Element	Certified value (μg/L)	Experiment value (μg/L)	Recovery rate (%)
As	17.226	15.6	90.56
Cd	1.767	1.56	88.29
Pb	5.546	3.8	68.52

4.2 Total concentrations of As, Cd and Pb in leafy vegetables
4.2.1 Arsenic (As)

Table 8 summarizes the total As concentrations found in all leafy vegetable samples. The total As concentrations (mg/kg) in “red coral (RC)”, “green oak (GO)” and “coral lettuce (CL)” which were grown by the soil cultivation ranged from 0.0064 to 0.1877, 0.0104 to 0.0800, 0.0207 to 0.2603, respectively. Meanwhile, the total As concentrations (mg/kg) in the RC, GO and CL which were hydroponically grown ranged from 0.0130 to 0.0990, 0.0054 to 0.0638, respectively. There were no significant differences ($p>0.05$) in As concentrations in different types of vegetables of the same cultivation method. Figure 6 shows the comparison of total arsenic (As) content of three leafy plants for soil and hydro cultivations. The average As concentrations (mg/kg) of RC, GO and CL for both cultivations were 0.0612± 0.0415, 0.0379 ± 0.0294 and 0.0606 ± 0.0419, respectively. The maximum As concentration (0.2603 mg/kg) in coral lettuce grown in the soil.

When compare the standard concentration of total As in leafy vegetables regulated by the Ministry of Public Health Thailand of 2 mg/kg (MOPH, 2020), the As concentrations in leafy vegetables cultivated by both methods were lower than the standard. In comparison to the WHO's standard of 0.1 mg/kg (World Health Organization, 2020), levels of As in RC and CL were higher than the standard except GO, which was grown by soil cultivation. It is interesting to find that the total As levels in all vegetables which were hydroponically grown were lower than both standards.

The findings of this study were also compared to those salad leafy vegetables reported in previous studies. According to Husaini et al. (2011), the maximum level of As (2.3±0.02 ug/g) were found in all salad leaves, all of which were approximately 2 times higher than the levels of As in salad leafy vegetables found in this study. In addition, the As concentrations of 1.50 to 4.14 mg/kg in lettuce grown at Philippi horticultural area as reported by Malan et al. (2015) were approximately 4 times higher than this study results. As levels in green vegetables reported by Kladsomboon et al. (2020) which were ranged from 0.08 to 0.24 mg/kg were comparable to the results of current study of 0.06 to 0.19 mg/kg. When considering organic and conventional cultivation practices, Xinyu et al. (2018) found comparable concentrations of As in leafy vegetables of 7.86 mg/kg in organic vegetables and 7.29 mg/kg in conventional vegetables. However, compared to the earlier results, the current study is significantly lower. Considering the cultivation area, Xu et al. (2015) discovered that leafy vegetables grown in industrial areas have a higher As concentration.

Table 8 Total concentrations of As, Cd and Pb in leafy vegetables grown by different cultivation methods

Vegetable	Concentration (mg/kg)					
	As		Cd		Pb	
	Soil	Hydro	Soil	Hydro	Soil	Hydro
Red coral (n=40)						
Minimum	0.0064	0.0130	0.0280	0.0223	0.0151	0.0215
Maximum	0.1877	0.0990	2.4862	0.4168	0.4656	0.2890
Mean	0.0612	0.0415	0.2599	0.1188	0.1878	0.0923
Median	0.0425	0.0429	0.1494	0.1092	0.1307	0.0760
SE	0.0072	0.0031	0.0742	0.0121	0.0209	0.0090
Green oak (n=40)						
Minimum	0.0104	0.0054	0.0402	0.0546	0.0174	0.0095
Maximum	0.0800	0.0638	0.9705	0.3875	0.5314	0.3091
Mean	0.0379	0.0294	0.2305	0.1602	0.1538	0.1447
Median	0.0359	0.0297	0.1453	0.1539	0.1273	0.1504
SE	0.0022	0.0017	0.0367	0.0123	0.0173	0.0104
Coral lettuce (n=40)						
Minimum	0.0207	0.0225	0.0216	0.0590	0.0411	0.0509
Maximum	0.2603	0.0943	0.9829	0.1989	0.3743	0.2385
Mean	0.0606	0.0419	0.1829	0.1121	0.1284	0.1217
Median	0.0477	0.0270	0.1189	0.1136	0.1393	0.1062
SE	0.0065	0.0041	0.0265	0.0046	0.0079	0.0102
MOPH standard	2mg/kg		0.2mg/kg		0.1mg/kg	
WHO standard	0.1mg/kg		0.2mg/kg		0.3mg/kg	

Remark: SE is standard error

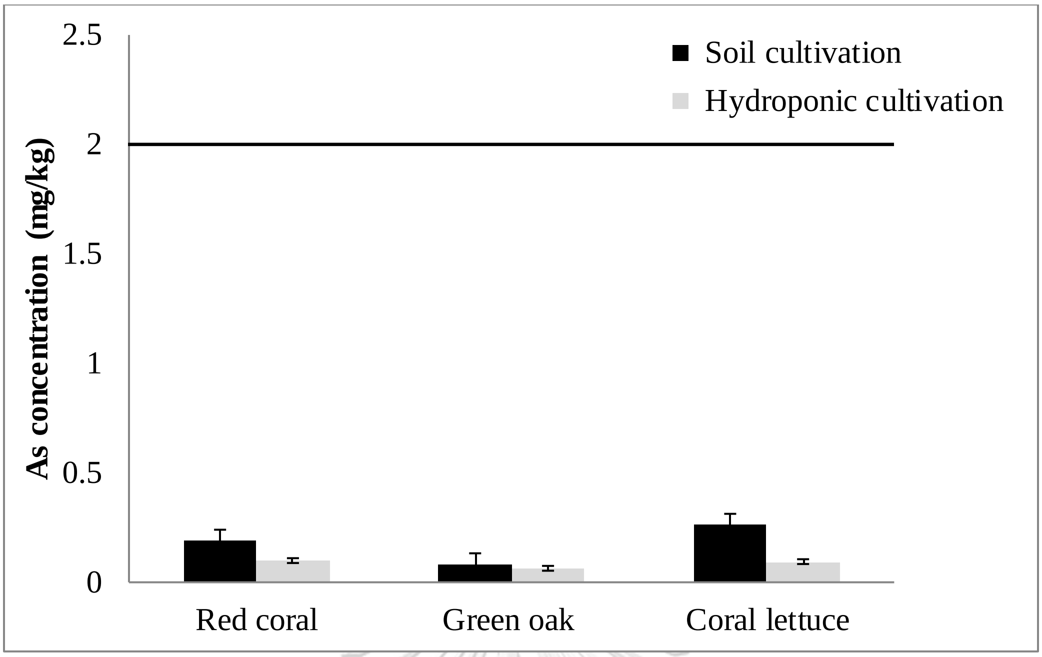

Figure 4 The comparisons of total As concentrations in different types of leafy vegetables grown by different cultivation methods

4.2.2 Cadmium (Cd)

Total Cd concentrations (mg/kg) in RC, GO, and CL which were grown by the soil cultivation were 0.0402 to 0.9705, 0.0280 to 2.4862, and 0.0216 to 0.9829, respectively. While the total Cd concentrations (mg/kg) in hydroponically grown RC, GO, and CL were 0.0223 to 0.4168, 0.0546 to 0.3875, and 0.0590 to 0.1989, respectively. For both cultivations, the Cd results of collected vegetables are summarized in Table 8. Among the three vegetables, interestingly, RC had the highest concentration for both cultivations. The results of statistical analyses indicated the significant higher total Cd concentrations in the RC which was cultivated in the soil than those of other vegetables ($p<0.05$). The average Cd concentrations (mg/kg) of RC, GO and CL were 0.2599 ± 0.1188, 0.2305 ± 0.1602, and 0.1829 ± 0.1121, respectively. Figure 5 shows the comparison of total Cd contents of three leafy plants for soil and hydro cultivations.

The standard Cd concentration in vegetables which is regulated by both Ministry of Public Health, Thailand (MOPH, 2020) and WHO/FAO, (World Health Organization, 2020) is 0.2mg/kg. When compared to the regulated limit, it was found that total Cd levels in RC, GO, and CL leafy vegetables cultivated in soil were higher than both standard. On the other hand, Cd levels in RC grown in soil culture were about 1.5 times greater than GO and CL. All vegetables cultivated in a hydroponic system had higher Cd level than the standard, except for CL.

The results of current study were compared to Cd levels in vegetables reported by earlier studies. Chunhabundit (2016) discovered that leafy vegetables which were obtained from Bangkok's major supermarkets and grocery stores, lettuce have higher Cd levels than spinach. However, the Cd concentrations in all vegetables were lower than the Codex's maximum Cd values of 0.2 mg kg^{-1}. In addition, comparing the contents of Cd in garden vegetables reported by (Kayee et al., 2018) , the ranges of Cd in leafy vegetables in the current study that were grown in soil were higher than the Thailand standard. According to Ali and Al-Qahtani (2012), Cd levels in lettuce varied from 0.92 to 4.13 mg/kg and 0.16 to 4.02 mg/kg. On the other hand, the average total Cd concentrations discovered in this study were lower than the average Cd concentrations reported in that study. Recently, Jalali and Karimi Mojahed (2020) reported that total Cd contents in greenhouse and open-field vegetables were in the range of 0.80 to 1.86 and 0.57 to 7.97 mg/kg which was around 5 times higher than the levels of Cd found in this study. The Cd concentration was found to be higher above the threshold level of 0.2 mg/kg as reported by World Health Organization (WHO).

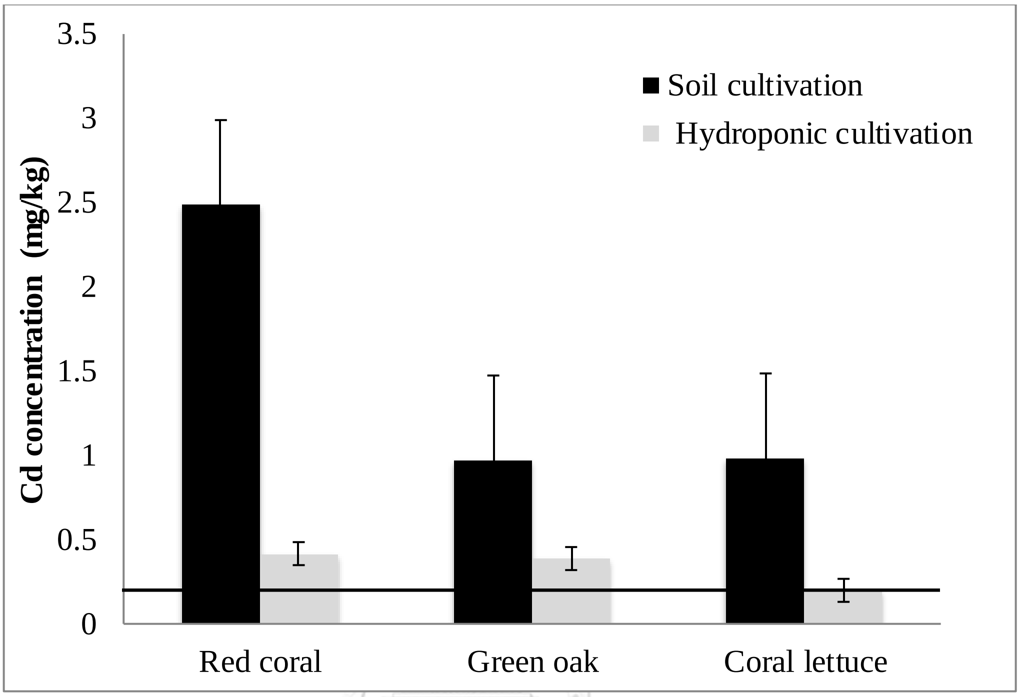

Figure 5 The comparisons of total Cd concentrations in different types of leafy vegetables grown by different cultivation methods

Plants' ability to absorb cadmium (Cd) relies on the soil characteristics, plant varieties, nutrients, and agronomic management and metal characteristics source (Fontes et al., 2014). Organic fertilizers derived from livestock manure and wastewater sludge are frequently applied in peri-urban farms. As a result, soil can get contaminated with Cd using agrochemicals, irrigation with sewage, livestock manures, and air deposition (Adams et al., 2004). Due to the frequent and significant application of fertilizers (organic or chemical) and irrigation in the vegetable greenhouse, urban and peri-urban agriculture is particularly susceptible to metal contamination (Zorrig et al., 2013). It has been reported that vegetables cultivated in peri-urban greenhouses are contaminated with Cd metals as a result of the excessive use of Cd-containing manures. The Cd content can be significantly immobilized through certain adsorption interactions with organic materials (Adams et al., 2004). The concentration of Cd in shoots and the effect of biochar on Cd accumulation in lettuce grown in a greenhouse were strongly positively associated (Zheng et al., 2017). According to Mourato et al. (2015), the increased Cd concentrations in plant shoots are mostly caused by the length of the roots, Cd translocation in the phloem and xylem, and Cd dilution in the shoots during growth. Plant growth is negatively impacted by cadmium, and its toxicity can be detected in both morphological and physiological impacts such as photosynthesis, respiration, transport and uptake of nitrogen and water (Ismael et al., 2019). Most investigations have shown a linear association between soil and plant Cd concentration (Min & Shi, 2018). Depending on multiple factors, it might be found that red coral grown in soil has the highest Cd concentration.

4.2.3 Lead (Pb)

The Pb contamination (mg/kg) in RC, GO and CL grown in soil ranged from 0.0151 to 0.4656, 0.0174 to 0.5314, and 0.0411 to 0.3743, respectively. The total Pb concentrations (mg/kg) in hydroponically RC, GO and CL were 0.0215 to 0.2890, 0.0095 to 0.3091, and 0.0509 to 0.2385, respectively. There were no significant differences ($p>0.05$) in Pb concentrations in different types of vegetables. The average Pb values (mg/kg) of RC, GO and CL were 0.1878 ± 0.0923, 0.1538 ± 0.1477, and 0.1284 ± 0.1217 for both cultivations. GO had the greatest amount (0.5314 mg/kg) compared to RC and CL among the three leafy plants growing in soil.

Figure 6 shows the comparison of total Pb contents of three leafy vegetables for soil and hydro cultivations.

When compare to the standard concentration of Pb in leafy vegetables regulated by the Ministry of Public Health Thailand of 0.1 mg/kg (MOPH, 2020), the total Pb concentrations in leafy vegetables cultivated by both methods were higher than the standard. In comparison to the WHO standard of 0.3 mg/kg (World Health Organization, 2020), levels of Pb in RC, GO and CL which was grown by soil cultivation were higher than the standard. With the exception of CL, the total Pb levels in vegetables which were hydroponically grown were within the WHO limit.

When comparing the Pb concentrations in leafy vegetables from the current study to the Pb levels in vegetables reported by previous research, the different results were found. For example, Farooq et al. (2008) discovered that Pb concentrations in lettuce and spinach grown in Pakistan's industrial districts ranged from 2.251 to 2.411 mg/kg, which was around 1.7 times higher than the Pb concentrations in the current study. According to Chabukdhara et al. (2016) and Hu (2010), soil-grown leafy vegetables contained Pb concentrations of 0.29 to 2.10 mg/kg and 0.51 to 2.18 mg/kg, respectively. The average Pb concentration in that study was higher than in this study, by approximately 1.5 times. When compared to the Pb concentrations of 0.18 to 1.79 mg/kg in leafy vegetables reported by Kananke et al. (2014), the results were approximately 1.3 times greater than the present results. Another study in China which was conducted by Hang et al. (2016) reported Pb contamination in vegetables grown on metal polluted soil with a concentration of 0.06 to 0.74 mg/kg. But the differences between the two outcomes are minimal. Pb concentrations in lettuce collected from fresh markets in Thailand's lower north ranged from 1.05 to 6.52 mg/kg (Wachirawongsakorn, 2016). The earlier results are significantly higher than the present results.

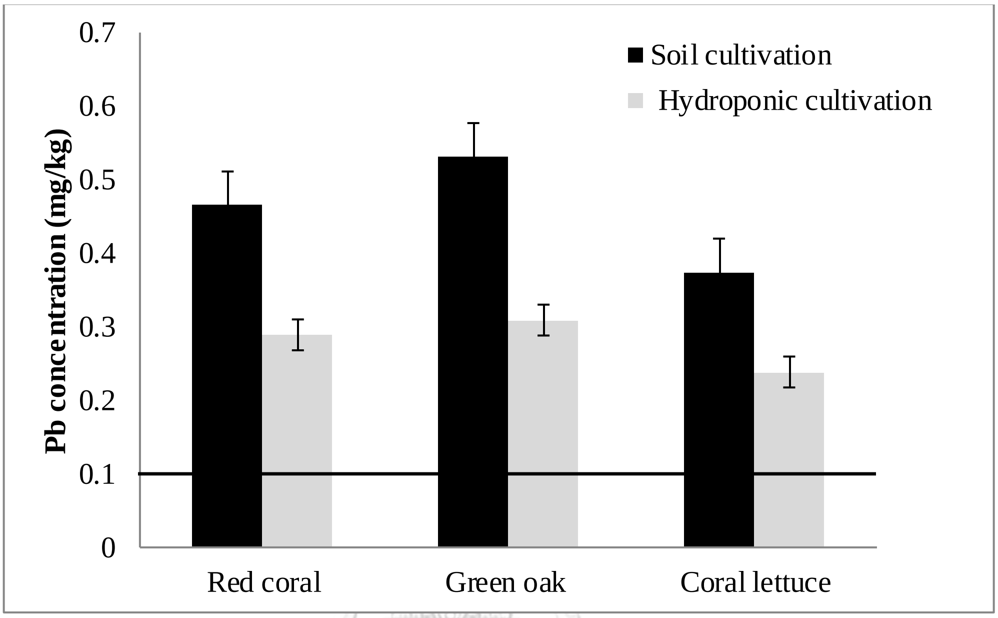

Figure 6 The comparisons of total Pb concentrations in different types of leafy vegetables grown by different cultivation methods

Based on the results of total concentrations of all metals of interests found in 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 coral lettuce, the maximum Cd concentration was found in the red coral, whereas the maximum Pb concentration was determined in the green oak. This study showed that soil-cultivated vegetables typically contained higher total concentrations of all metals of interests than those of the hydroponically grown vegetables. This can be explained by the reason that soil generally contains both essential and non-essential metals including As, Cd and Pb. There are several studies reported about metals 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 soil quality standard, Thailand (Kladsomboon et al., 2020). According to Chang et al. (2014a), all of the total As, Cd, and Pb concentrations in agricultural soils in South China were greater than background metal levels in Guangdong, China, and ranged from 0.68 to 105, 0.01 to 0.69, and 3.42 to 140 (mg/kg dry soil), respectively. Moreover, Marinova (2003) discovered that Bursa, Turkey agricultural soil contained Cd and Pb concentrations (g/kg) of 0.1 to 8.7 and 33.4 to 163.3, respectively. In addition, it might be the geological properties of soils and parent rocks, as well as anthropogenic activity that can elevate the metal concentrations in soil to become higher than the natural levels.

In Bangkok, it has been established that vehicle emissions can contaminate soil with trace metals such as Cd and Pb, which are frequently present in high concentrations in road dust. The primary causes are tire abrasion and fuel combustion Krailertrattanachai et al. (2019). Around 3,920,000 (Economics, 2018) and 198,000 (Economics, 2017) tons of fertilizers and insecticides are used annually in Thailand, respectively. Therefore, repetitive chemical application during agricultural activities may result in the accumulation of heavy metals in the environment (Kuziemska et al., 2016). Phosphate fertilizers are discovered to be important sources of As in some developing countries like Thailand (Kladsomboon et al., 2020). After spraying pesticides and herbicides, elevated amounts of Cd and Pb are discovered (Ning et al., 2017). According to Ning et al. (2017), the continual use of organic fertilizer in vegetable cultivation has led to increasing soil concentrations of Zn, Cd, and Cr. Due to the extensive usage of chemical fertilizer produced through the biosolids process on agricultural land, atmospheric soil pollution is also rising in Bangkok's suburbs (Zarcinas et al., 2004). According to Ruchirawat et al. (2007), when heavy metals contaminated the soil and accumulated over time, the metals might be transferred from the soil by plants, microorganisms, animals, and mammals. People in Bangkok were exposed to the long-term hazards as a result of eating contaminated food, particularly young children (Ruchirawat et al., 2007). By using metal-containing pesticides and fertilizers, it is possible for some of the harmful compounds in the environment to reach the air, surface water, or soil (Pamonpol & Tokhun, 2019). The danger chemicals can be entered into water by direct and indirect ways. Acid rain is able to increase metal mobilization in soil to water ecosystems by substituting cations in the acidic water and increasing the concentrations of the cations (Pb and Cd) in the soil-water system (Zarcinas et al., 2004).

Furthermore, Huang and Jin (2008) reported that Cd and Pb were common anthropogenic elements in the urban environment as the result of the excessive usage of chemical fertilizers and organic manures for open vegetable fields and greenhouse vegetable fields which finally lead to the buildup of metals in soils. Additionally, harmful metal pollutants like Cd are included in superphosphate and lime fertilizers. These toxins can accumulate in the soil, be absorbed by plants, and spread to other organisms through the food chain (Shao & Ji, 2008). In addition, it was found that the lower pH in soil after nitrogen and phosphate fertilizer applications on agricultural fields can cause metal desorption from the soil matrix and resulted in the higher concentrations of As, Cd and Pb in vegetables (Wangstrand et al., 2007). In China, the main sources of metal pollution entering agricultural soils may be irrigation water and industrial pollutants (Ye et al., 2015). According to the results of principal component analysis (PCA), parent rocks were also discovered to be the primary regulators of As concentrations, but artificial activities such as automobiles, industrial emissions, and waste water irrigation, had an impact on Cd and Pb levels (Ping et al., 2011). Atafar et al. (2010) also reported that after applying inorganic fertilizers or liquid and soil manure (compost or sludge) to both uncovered and greenhouse vegetable crops, it is possible that the higher metal concentrations in agricultural soils can be found. In addition, leaf vegetables have more chance of being exposed to outside contaminants from precipitation and soil dust due to their wide leaf area (Gezahegn et al., 2017). The plant's growth stages also influenced metal accumulations in the leaves through the absorption of the soil by the roots and air pollution by the leaves (Souri et al., 2019). Chang et al. (2014b) also found the multiple factors which can influence metal accumulation in vegetables tissues including soil qualities (e.g., soil pH, organic matter, clay content, and metal concentration), plant parameters (e.g., plant type), and other environmental conditions (e.g., atmosphere and industrial pollution). Moreover, agricultural soil pollution by metals is one of the most pressing challenges in China, according to Zhao et al. (2015), but it also occurs in Pakistan (Jadoon & Malik, 2019), South Korea (Kang et al., 2019), India (Kaur et al., 2019) and Serbia (Spahić et al., 2019). The research described above show that agro-ecosystem contamination is a worldwide problem that has an impact on healthy vegetable production.

4.3 Exposure assessment of As, Cd and Pb through consumption of salad leafy vegetables

The exposure rates of three elements (As, Cd, and Pb) were calculated for three different exposure groups according to the concentrations of each metal in vegetables and the ingestion rate of the Thai population. The average body weights used in this present study were obtained from the National Health Survey (NHS) for Thai people (WHO, 2008-2009). The calculation of the exposure rate for this study was divided the population into three groups: children, adolescents, and adults. Table 9 and Table 10 summarizes the total body weights of Thai population and the values of all variables used for the calculations of exposure rates used in this study, respectively.

Table 9 Data on the body weights (kg) of Thai population

Population		Min	Max	Mean	SD
Children (3 to 12.9 years)	All population	15.00	85.20	32.29	11.75
	Male	15.00	85.20	32.79	11.99
	Female	15.00	69.50	31.84	11.53
Adolescents (13 to 17.9 years)	All population	25.70	106.40	52.78	14.90
	Male	29.70	101.20	53.44	14.57
	Female	25.70	106.20	52.11	15.32
Adults (18 to 64.9 years)	All population	26.10	133.10	70.36	13.46
	Male	42.10	133.10	71.06	13.46
	Female	26.10	117.60	69.65	12.90

Table 10 Summary of variable values used for exposure rates of As, Cd and Pb via vegetables consumption

Population	Value of risk assessment variable	
	Body weight (kg)	**Consumption per capita (kg/day)**
Children (3 to 12.9 years)	32.29	0.00041
Adolescents (13 to17.9 years)	52.78	0.00086
Adults (18 to 64.9 years)	70.36	0.00138

Table 11 and Table 12 summarize the As, Cd, and Pb exposure rates in different population groups from the consumption of different types of vegetables.

The exposure rates of the metals and the hazard quotient (HQ) were computed based on the consumption rate of leafy vegetables to estimate the potential health effects to the Thai population.

4.3.1 Arsenic (As) exposure via leafy vegetables ingestion

Tables 11 and 12 summarize the average daily dose (ADD) of As exposure from different leafy vegetable consumptions which are grown by different cultivation methods. According to the results of statistical analyses, the ADD of As exposure are not significantly different ($p>0.05$). The average daily dose of As exposure in adults were higher than those of adolescents and children. The exposure rates of As through the daily consumption of leafy vegetables grown in soil in children, adolescents, and adults were 3.31×10^{-5}, 2.12×10^{-5}, and 2.40×10^{-4} mg/kg·day, respectively. While As exposure levels (mg/kg·day) from regular consumption of hydroponically grown vegetables were 1.26×10^{-5}, 8.07×10^{-6}, and 9.13×10^{-5}, respectively. Moreover, based on the results of different age groups, adults were the group of population who have expose to the highest level of As from leafy vegetables consumption from the daily basis. The order of As exposure rates was adults > adolescents > children since the consumption rate was higher in adult.

When compare the average daily dose of As exposure from the consumption of vegetables concerning cultivated by different cultivation methods, it was found that CL consumption which was grown in soil, showed the highest As exposure rate among the three vegetables grown by the same cultivation method. The orders of As exposure rates (mg/kg·day) in the adult population who consume soil cultivated vegetables were in the following orders: 2.40×10^{-4} for CL, 1.73×10^{-4} for RC, and 7.37×10^{-5} for GO.

Aside from this study, As exposure rates through leafy vegetable consumption have been reported in various countries. Consuming leafy vegetables grown in wastewater irrigation zones resulted in As exposure rates of 1.3×10^{-6} and 2.9×10^{-6} (mg/kg·day) (Jolly et al., 2013). The As exposure rates at this level were approximately 2.4 times lower than the As exposure rates in Thai adults in the current study. Sultana et al. (2017) reported that the average As exposure levels from vegetable intakes in Bangladesh were 3.9×10^{-5}, 6.3×10^{-5}, and 8.4×10^{-5} (mg/kg·day), all of which were lower than the present results by approximately 1.5 times. As exposure through lettuce ingestion of (mg/kg·day) 2.3×10^{-6} and 1.1×10^{-6} as reported by (Salehipour et al., 2015) were lower than this present study by approximately 2.5 times. Additionally, Bempah and Ewusi (2016) discovered that eating vegetables grown in polluted soil near gold mines resulted in the As exposure rate ranging from 2.20×10^{-3} to 2.40×10^{-6} mg/kg·day. However, compared to earlier findings, the As exposure levels from this study were approximately 2 times lower. Hadayat et al.

(2018) found that the consumption of organic and conventional veggies from a Florida supermarket can cause the As exposure levels in adults of 2.3 x 10^{-4}, and 1.3 x 10^{-4}, 1.6 x 10^{-4} mg/kg·day. When compared to the most current outcomes of As exposure, the previous results were not different than those discovered in this study.

4.3.2 Cadmium (Cd) exposure via leafy vegetables ingestion

The average daily dose (ADD) of Cd exposure from the consumption of various leafy vegetables cultivated using different methods is shown in Tables 11 and 12. Children, adolescents, and adults were all exposed to Cd at rates of 3.16×10^{-4}, 2.0×10^{-4}, and 2.29×10^{-3} mg/kg·day, respectively, through the daily consumption of green vegetables cultivated in soil. The regular consumption of hydroponically grown vegetables resulted in Cd exposure levels (mg/kg·day) of 5.29×10^{-5}, 3.40×10^{-5}, and 3.84×10^{-4}, respectively. For statistical analyses, the Cd exposure in adults was approximately 2 times higher than the others ($p<0.05$). According to the results of different age groups, adults were exposed to Cd at higher rates than adolescents and children from leafy vegetables on a regular basis as the intake was greater in adults.

When comparing the average daily dose of Cd exposure from the consumption of vegetables cultivated by different cultivation methods, it was found that RC consumption which was grown in soil showed the highest Cd exposure rate among the three vegetables grown by the same cultivation method. The rates of adult population's Cd exposure for soil-grown vegetables were in the ranges of 2.29×10^{-3} for RC, 9.06×10^{-4} for CL, and 8.95×10^{-4} for GO.

Different results were achieved when compared to prior studies, especially on Cd exposure rates. The present Cd exposure rate was approximately 1.7 times higher than the Cd exposure rate through the consumption of vegetables of 5.98×10^{-4} mg/kg·day which was reported by Cao et al. (2010). According to Guerra et al. (2012b), Cd exposure levels (mg/kg·day) through the consumption of vegetables like iceberg lettuce, smooth lettuce and crisp head lettuce were 4.1×10^{-5}, $3.2 \times 10^{-5,}$ 4.6×10^{-5}, respectively. When comparing both results, the exposure rates of Cd in this study were approximately 2 times higher than the past results. Moreover, the daily exposure of Cd from vegetables ingestion in India was 4.77×10^{-4} mg/kg·day for adults and 5.48×10^{-4} mg/kg·day for children as reported by Chauhan and Chauhan (2014). But the reported results were lower the Cd exposure rates than this study. The Cd exposure rate found from lettuce consumption grown in wastewater irrigation in Dubai ranged from 4.69×10^{-4}, 5.0×10^{-4}, 5.39×10^{-4} mg/kg·day (Hussain & Qureshi, 2020), while the present study results were higher than those results. Furthermore, Orisakwe et al. (2012) and Ćwieląg-Drabek et al. (2020) discovered that the consumption of vegetables grown in polluted soil near mining areas resulted in Cd exposure rates ranging from 6.0×10^{-3} to 7×10^{-3} mg/kg·day and $4.5x10^{-3}$ to $7x10^{-3}$ mg/kg·day. However, compared to earlier findings, the Cd exposure levels from this study were lower than those studies. In contrast, the Cd exposure from daily consumption of lettuce and spinach cultivated in contaminated soil was $3.9 \text{ x } 10^{-3}$ and $4.6 \text{ x } 10^{-3}$ mg/kg·day which was reported by Ahmed et al. (2022) were approximately 1.5 times greater than to the present study.

4.3.3 Lead (Pb) exposure via leafy vegetables ingestion

Tables 11 and 12 show the average daily dosage (ADD) of Pb exposure from the ingestion of various green vegetables grown in different cultivation methods. Children, adolescents, and adults were exposed to Pb at rates of 6.75×10^{-5},4.33×10^{-5}, and 4.90×10^{-4} mg/kg·day, respectively, through the daily ingestion of green vegetables grown in soil. Pb exposure levels in children, adolescents, and adults of 3.92×10^{-5}, 2.52×10^{-5}, and 2.85×10^{-4} mg/kg·day were attained via regular consumption of hydroponically grown vegetables. When compared to different age groups, adults' Pb exposure was statistically significant higher ($p < 0.05$) than adolescents and children since children and adolescents consumed fewer leafy vegetables than adults.

When comparing the vegetables cultivated using the different methods, the ingestion of GO grown in soil showed the highest Pb exposure. For soil-grown vegetables, the Pb exposure rates for the adult population ranged from 4.90×10^{-4} for GO, 4.29×10^{-4} for RC, and 3.45×10^{-4} for CL. The Pb exposure rates to population from soil-grown vegetables always higher than those of the Pb exposure rates from hydroponically grown ones.

Different Pb exposure results were found when compared to those from previous studies. According to Nedelescu et al. (2017), Pb exposure from intake of vegetables cultivated in the industrial regions of Romania was 3.5×10^{-3} mg/kg·day. The earlier results were higher than around 3 times the present results. Although the levels of Pb exposed through regular vegetable ingestion in India were 1.58×10^{-3} mg/kg·da for adults and 1.82×10^{-3} mg/kg·day for children (Chauhan & Chauhan, 2014), the current study was lower than those results. Cherfi et al. (2015) and Orisakwe et al. (2012) discovered that Pb exposure from vegetables consumption purchased from the Nigeria supermarket was 4.59×10^{-4} to1.87×10^{-5} mg/kg·day and 1.4×10^{-3} to 6.2×10^{-4} mg/kg·day. The level of Pb exposure rate was higher than this study by approximately 1.3 times. Pb exposure rates from consumption of green vegetables cultivated near mining and smelting facilities ranged from $5.7\text{x}10^{-4}$ and $1.8\text{x}10^{-5}$ mg/kg·day (Ćwieląg-Drabek et al., 2020) when compared, however the earlier results were greater than the later ones. Salehipour et al. (2015) reported the Pb exposure from daily lettuce consumption was 1.1×10^{-5} mg/kg·day. The levels of Pb exposure rate via vegetables from Iran and the Thai adult population were different for both studies.

Table 11 Average daily dose (ADD) (mg/kg·day) of As, Cd and Pb exposure in different population via different types of leafy vegetables grown in soil cultivation

Element	Population		Vegetable		
			Red coral	**Green oak**	**Coral lettuce**
As	Children (3 to 12.9 years)	Min	8.13×10^{-7}	1.32×10^{-6}	2.63×10^{-6}
		Max	2.38×10^{-5}	1.02×10^{-5}	3.31×10^{-5}
		Mean	7.77×10^{-6}	4.81×10^{-6}	7.69×10^{-6}
		Median	5.40×10^{-6}	4.56×10^{-6}	6.06×10^{-6}
		SE	9.14×10^{-7}	2.79×10^{-7}	8.25×10^{-7}
	Adolescents (13 to 17.9 years	Min	5.21×10^{-7}	8.47×10^{-7}	1.69×10^{-6}
		Max	1.53×10^{-5}	6.52×10^{-6}	2.12×10^{-5}
		Mean	4.99×10^{-6}	3.09×10^{-6}	4.94×10^{-6}
		Median	3.46×10^{-6}	2.92×10^{-6}	3.89×10^{-6}
		SE	5.87×10^{-7}	1.79×10^{-7}	5.30×10^{-7}
	Adult (18 to 64.9 years)	Min	5.90×10^{-6}	9.59×10^{-6}	1.91×10^{-5}
		Max	1.73×10^{-4}	7.37×10^{-5}	2.40×10^{-4}

		Mean	5.64×10^{-5}	3.49×10^{-5}	5.59×10^{-5}
		Median	3.92×10^{-5}	3.31×10^{-5}	4.40×10^{-5}
		SE	6.64×10^{-6}	2.03×10^{-6}	5.99×10^{-6}
Cd	Children (3 to 12.9 years)	Min	3.56×10^{-6}	5.10×10^{-6}	2.74×10^{-6}
		Max	3.16×10^{-4}	1.23×10^{-4}	1.25×10^{-4}
		Mean	3.30×10^{-5}	2.93×10^{-5}	2.32×10^{-5}
		Median	1.90×10^{-5}	1.84×10^{-5}	1.51×10^{-5}
		SE	9.42×10^{-6}	4.66×10^{-6}	3.36×10^{-6}
	Adolescents (13 to 17.9 years	Min	2.28×10^{-6}	3.28×10^{-6}	1.76×10^{-6}
		Max	2.0×10^{-4}	7.91×10^{-5}	8.01×10^{-5}
		Mean	2.12×10^{-5}	1.88×10^{-5}	1.49×10^{-5}
		Median	1.22×10^{-5}	1.18×10^{-5}	9.69×10^{-6}
		SE	6.05×10^{-6}	2.99×10^{-6}	2.16×10^{-6}
	Adult (18 to 64.9 years)	Min	2.58×10^{-5}	3.71×10^{-5}	1.99×10^{-5}
		Max	2.29×10^{-3}	8.95×10^{-4}	9.06×10^{-4}
		Mean	2.40×10^{-4}	2.12×10^{-4}	1.69×10^{-4}
		Median	1.38×10^{-4}	1.34×10^{-4}	1.10×10^{-4}
		SE	6.84×10^{-5}	3.38×10^{-5}	2.44×10^{-5}
Pb	Children (3 to 12.9 years)	Min	1.92×10^{-6}	2.21×10^{-6}	5.22×10^{-6}
		Max	5.91×10^{-5}	6.75×10^{-5}	4.75×10^{-5}
		Mean	2.38×10^{-5}	1.95×10^{-5}	1.63×10^{-5}
		Median	1.66×10^{-5}	1.62×10^{-5}	1.77×10^{-5}
		SE	2.65×10^{-6}	2.20×10^{-6}	1.00×10^{-6}
	Adolescents (13 to 17.9 years)	Min	1.23×10^{-6}	1.42×10^{-6}	3.35×10^{-6}
		Max	3.79×10^{-5}	4.33×10^{-5}	3.05×10^{-5}
		Mean	1.53×10^{-5}	1.25×10^{-5}	1.05×10^{-5}
		Median	1.06×10^{-5}	1.04×10^{-5}	1.13×10^{-5}
		SE	1.70×10^{-6}	1.41×10^{-6}	6.44×10^{-7}
	Adult (18 to 64.9 years)	Min	1.39×10^{-5}	1.60×10^{-5}	3.79×10^{-5}
		Max	4.29×10^{-4}	4.90×10^{-4}	3.45×10^{-4}
		Mean	1.73×10^{-4}	1.42×10^{-4}	1.18×10^{-4}

		Median	1.20×10^{-5}	1.17×10^{-4}	1.28×10^{-4}
		SE	1.93×10^{-5}	1.59×10^{-5}	7.28×10^{-6}

Table 12 Average daily dose (ADD) (mg/kg·day) of As, Cd and Pb exposure in different population via different types of leafy vegetables grown in hydroponic cultivation

Element	Population		Vegetable		
			Red coral	Green oak	Coral lettuce
	Children	Min	1.65×10^{-6}	6.86×10^{-7}	2.86×10^{-6}
	(3 to 12.9 years)	Max	1.26×10^{-5}	8.10×10^{-6}	1.20×10^{-5}
		Mean	5.27×10^{-6}	3.73×10^{-6}	5.32×10^{-6}
		Median	5.45×10^{-6}	3.77×10^{-6}	3.43×10^{-6}
		SE	3.94×10^{-7}	2.16×10^{-7}	5.21×10^{-7}
	Adolescents	Min	1.06×10^{-6}	4.40×10^{-7}	1.83×10^{-6}
As	(13 to 17.9 years)	Max	8.07×10^{-6}	5.20×10^{-6}	7.68×10^{-6}
		Mean	3.38×10^{-6}	2.40×10^{-6}	3.41×10^{-6}
		Median	3.50×10^{-6}	2.42×10^{-6}	2.20×10^{-6}
		SE	2.53×10^{-7}	1.38×10^{-7}	3.34×10^{-7}
	Adult	Min	1.20×10^{-5}	4.98×10^{-6}	2.07×10^{-5}
	(18 to 64.9 years)	Max	9.13×10^{-5}	5.88×10^{-5}	8.69×10^{-5}
		Mean	3.83×10^{-5}	2.71×10^{-5}	3.86×10^{-5}
		Median	3.95×10^{-5}	2.7×10^{-5}	2.49×10^{-5}
		SE	2.86×10^{-6}	1.57×10^{-6}	3.78×10^{-6}
	Children	Min	2.83×10^{-6}	6.93×10^{-6}	7.49×10^{-6}
	(3 to 12.9 years)	Max	5.29×10^{-5}	4.92×10^{-5}	2.53×10^{-5}
		Mean	1.51×10^{-5}	2.03×10^{-5}	1.42×10^{-5}
		Median	1.39×10^{-5}	1.95×10^{-5}	1.44×10^{-5}
		SE	1.54×10^{-6}	1.56×10^{-6}	5.84×10^{-7}
Cd	Adolescents	Min	1.82×10^{-6}	4.45×10^{-6}	4.81×10^{-6}
	(13 to 17.9 years	Max	3.40×10^{-5}	3.16×10^{-5}	1.62×10^{-5}
		Mean	9.68×10^{-6}	1.31×10^{-5}	9.13×10^{-6}

		Median	8.90×10^{-6}	1.25×10^{-5}	9.26×10^{-6}
		SE	9.86×10^{-7}	1.00×10^{-6}	3.75×10^{-7}
	Adult (18 to 64.9 years)	Min	2.06×10^{-5}	5.03×10^{-5}	5.44×10^{-5}
		Max	3.84×10^{-4}	3.57×10^{-4}	1.83×10^{-4}
		Mean	1.10×10^{-4}	1.48×10^{-4}	1.03×10^{-4}
		Median	1.01×10^{-4}	1.42×10^{-4}	1.05×10^{-4}
		SE	1.12×10^{-5}	1.13×10^{-5}	4.24×10^{-6}
Pb	Children (3 to 12.9 years)	Min	2.73×10^{-6}	1.21×10^{-6}	6.46×10^{-6}
		Max	3.67×10^{-5}	3.92×10^{-5}	3.03×10^{-5}
		Mean	1.17×10^{-5}	1.84×10^{-5}	1.55×10^{-5}
		Median	9.65×10^{-6}	1.91×10^{-5}	1.35×10^{-5}
		SE	1.14×10^{-6}	1.32×10^{-6}	1.30×10^{-6}
	Adolescents (13 to 17.9 years)	Min	1.75×10^{-6}	7.74×10^{-7}	4.15×10^{-6}
		Max	2.35×10^{-5}	2.52×10^{-5}	1.94×10^{-5}
		Mean	7.52×10^{-6}	1.18×10^{-5}	9.91×10^{-6}
		Median	6.19×10^{-6}	1.23×10^{-5}	8.65×10^{-6}
		SE	7.33×10^{-7}	8.47×10^{-7}	8.31×10^{-7}
	Adult (18 to 64.9 years)	Min	2.73×10^{-6}	8.76×10^{-6}	4.69×10^{-5}
		Max	3.67×10^{-5}	2.85×10^{-4}	2.20×10^{-4}
		Mean	1.17×10^{-5}	1.33×10^{-4}	1.12×10^{-4}
		Median	9.65×10^{-6}	1.39×10^{-4}	9.79×10^{-5}
		SE	1.14×10^{-6}	9.59×10^{-6}	9.40×10^{-6}

4.4 Non-carcinogenic health risks from leafy vegetables consumption

4.4.1. Arsenic (As)

Tables 13 to 14 and Figures 7 to 8 provide an overview of the non-carcinogenic risks of As exposure by age group. The HQ values of As exposure through the consumption of leafy vegetables grown in soil in children, adolescents, and adults were 0.11, 0.07 and 0.80, respectively. While HQ values of As exposure from regular consumption of hydroponically grown vegetables were 0.04, 0.03 and 0.29, respectively. The HQ values were ranked in the following order by age group: adults > children > adolescents. The HQ of values of As in adults were significantly higher than ($p<0.05$) children and adolescents.

When compare the HQ values of As exposure from the consumption of vegetables cultivated by different cultivation methods, it was found that CL consumption which was grown in soil showed the highest As exposure rate among the three vegetables grown by the same cultivation method. As a result, if the exposure levels were high, the HQ value could be influenced as well. In the adult population who consumes vegetables grown in soil, the HQ values of As exposure rates were in the following orders: 0.80 for CL, 0.58 for RC, and 0.25 for GO.

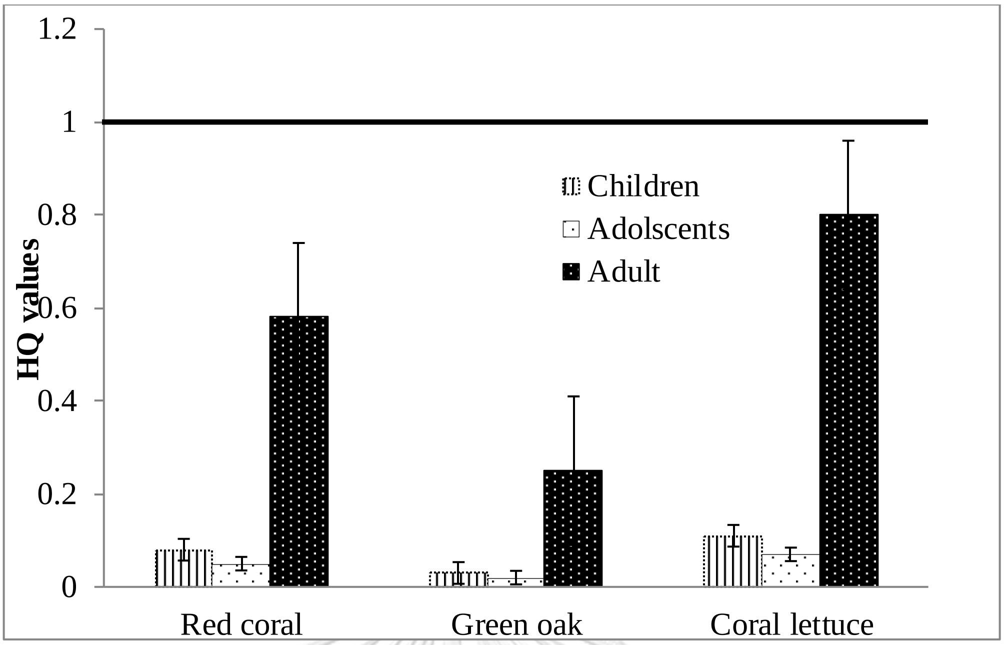

Figure 7 Levels of hazard quotient from the consumption of different soil cultivated vegetables

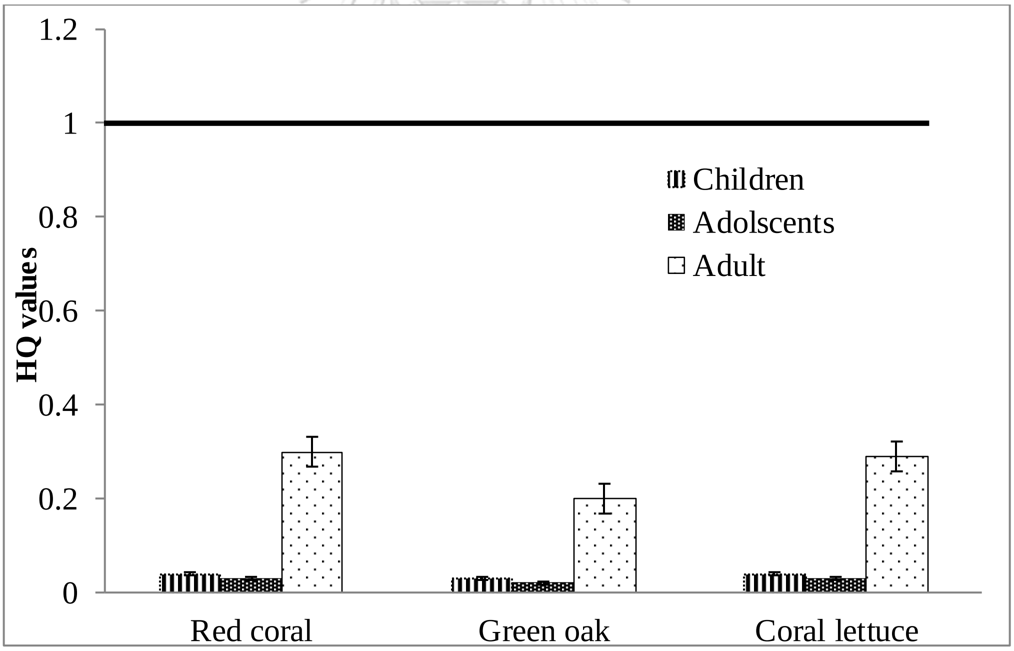

Figure 8 Levels of hazard quotient from the consumption of different hydroponically vegetables

The different results were obtained when comparing the findings of this study to those of previous investigations. When compared to the HQ values of As exposure in salad leaves, which ranged from 0.01 to 4.31 reported by Kladsomboon et al. (2020), the current HQ values were approximately 3.5 times lower than those previous results. Wang et al. (2012) discovered that HQ values of As exposure of 0.55 to 2.3 in the Chinese population who consumed vegetables grown in polluted soil near industrial zones and the results were approximately 1.5 times higher than the current study. According to Shaheen et al. (2016) and Hadayat et al. (2018), the regular consumption of vegetables obtained from supermarkets resulted in HQ values of As ranging from 0.02 to 0.11 for children and 0.03 to 0.13 for adults . The results of the current study are not significantly different from the earlier ones.

4.4.2. Cadmium (Cd)

The non-carcinogenic risks of HQ values Cd exposure by age group are summarized in Tables 13 to 14 and Figures 9 to 10. Children, adolescents, and adults had the Cd exposure of HQ values were 0.32, 0.20, and 2.29, respectively, from eating leafy vegetables cultivated in soil. The HQ values for Cd exposure from consumption of hydroponically grown vegetables were 0.05, 0.03, and 0.38, respectively. By age category, the HQ values were rated as follows: adults > children > adolescents. In comparison to children and adolescents, adults' HQ values for Cd were approximately 2 times greater ($p<0.05$) than other groups of population. In addition, HQ levels of Cd significantly higher in the adult population who consumed RC compared to GO and CL grown by the soil cultivation. The HQ values of Cd exposure rates were in the following orders in the adult population: 2.29 for RC, 0.91 for CL, and 0.89 for GO.

The Cd exposure rates discovered in this study are significantly lower than the HQ values of Cd exposure in Chinese population reported by Chang et al. (2014b) and Zheng et al. (2007). Due to the vegetable consumption, HQ values of Cd ranged from 5.79 to 9.90 for children and 7.6 to 14.0 for adults in both studies, and they are significantly higher than the Chinese norm. The HQ values of Cd levels were 0.11 and 0.13 for children and adults via salad vegetable consumption (Guerra et al., 2012a). The current HQ values were approximately 2.2 times greater than the previous results. According to Shaheen et al. (2016), HQ levels of Cd in mustard leafy vegetables ranged from 0.005 to 0.04 in Bangladesh, indicating that the results were lower than the current findings. Mahmood and Malik (2014) reported that the Cd HQ values of children and adults who consumed spinach and coriander cultivated in wastewater-irrigated areas were 0.07 to 0.13 and 0.10 to 0.28, respectively. However, the results of this study were approximately 2 times higher than those results. In Pakistan, Cd levels ranging from 0.02 to 0.05 were found from the consumption of watercress green vegetables grown in swampy areas, all of which were lower than this present results (Khan et al., 2022).

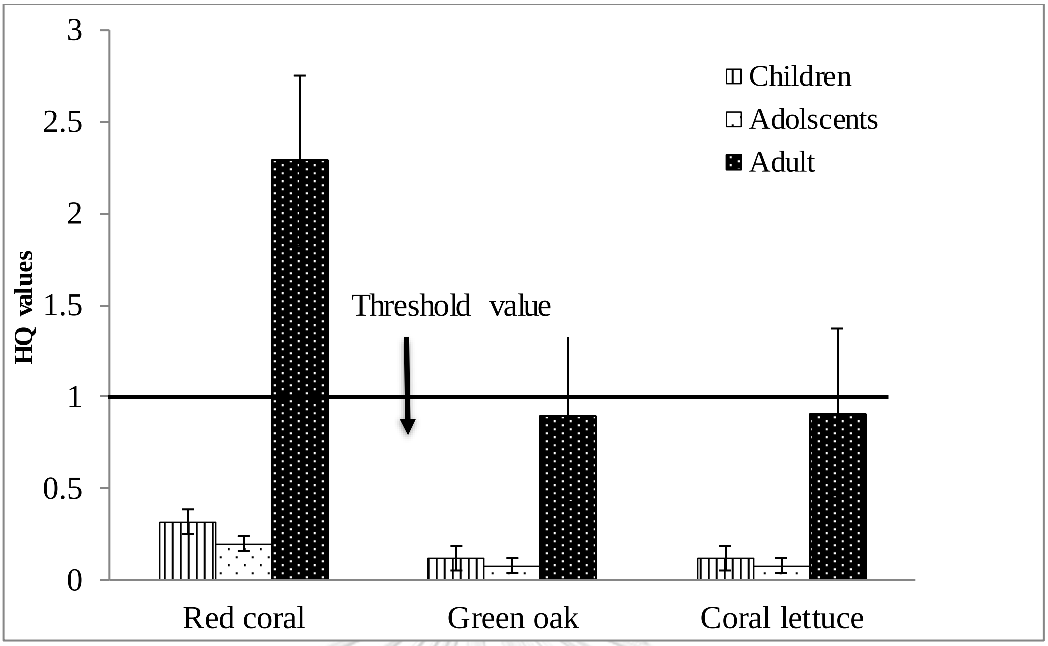

Figure 9 Levels of hazard quotient from the consumption of different soil cultivated vegetables

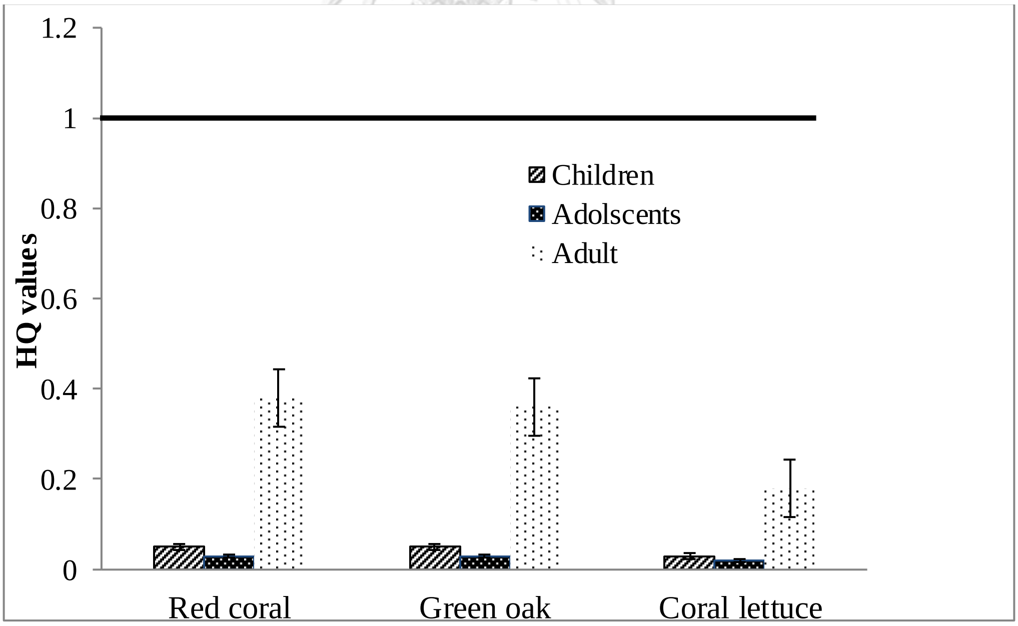

Figure 10 Levels of hazard quotient from the consumption of different hydroponically grown vegetables

4.4.3 Lead (Pb)

Tables 13 to 14 and Figures 11 to 12 summarize the non-carcinogenic risks of exposure to high levels of Pb by age group. Pb exposure to HQ levels from eating soil-grown leafy vegetables was 0.02, 0.01, and 0.14 in children, adolescents, and adults. The HQ values for Pb exposure from consumption of vegetables grown hydroponically were 0.01 and 0.08, respectively. The HQ values of Pb in the adult population were not significantly different ($p>0.05$) from those of children and adolescents. But the higher HQ levels of Pb found in GO vegetables consumption which are grown in soil. The HQ values of Pb exposure rates were in the following order in the adult population: 0.14 for GO, 0.12 for RC, and 0.10 for CL.

According to Cao et al. (2010), the HQ values of Pb were 0.12 to 0.25 in the Chinese population through the consumption of garden vegetables. Those results are not different from the current results. The HQ of Pb 1.76 to 6.17 exposure to local children indicates that the previous results were significantly higher than in this study (Cai et al., 2019). Moreover, Pb levels in highly consumed vegetables in Ethiopia ranged from 0.04 to 0.09 for the adult population according to Gebrekidan et al. (2013). There was no difference between the current and previous results. Mahmood and Malik (2014) discovered that children and adults who consumed vegetables grown in wastewater-irrigated areas had Pb HQ levels of 0.04 to 0.11 and 0.04 to 0.06, all of which are almost similar to this study. Pb levels in loose leaf lettuce ranged from 0.04 to 0.09 according to Shaheen et al. (2016).When comparing the results, both results are not much different from the range.

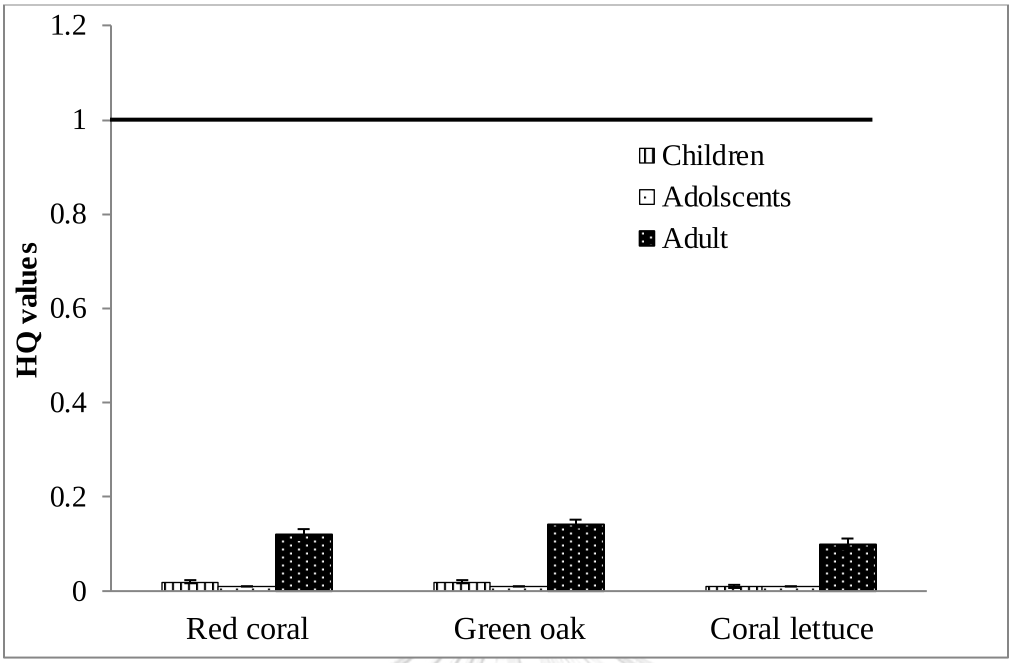

Figure 11 Levels of hazard quotient from the consumption of different soil grown vegetables

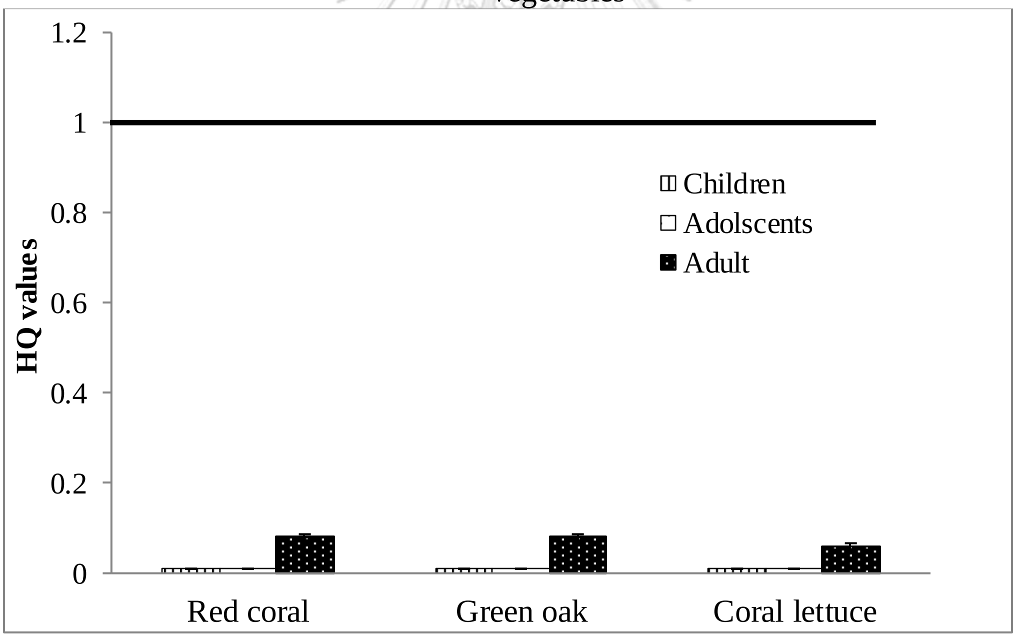

Figure 12 Levels of hazard quotient from the consumption of different hydroponically grown vegetables

Table 13 Values of hazard quotient of metal exposure from the consumption of soil grown vegetables

Element	Population		Vegetable		
			Red coral	Green oak	Coral lettuce
As	Children (3 to12.9 years)	Min	0.00	0.00	0.01
		Max	0.08	0.03	0.11
		Mean	0.03	0.02	0.03
		Median	0.02	0.02	0.02
		SE	0.00	0.00	0.00
	Adolescents (13 to 17.9 years)	Min	0.00	0.00	0.01
		Max	0.05	0.02	0.07
		Mean	0.02	0.01	0.02
		Median	0.01	0.01	0.01
		SE	0.00	0.00	0.00
	Adults (18 to 64.9 years)	Min	0.02	0.03	0.06
		Max	0.58	0.25	0.80
		Mean	0.19	0.12	0.19
		Median	0.13	0.11	0.15
		SE	0.02	0.01	0.02
Cd	Children (3 to12.9 years)	Min	0.00	0.01	0.00
		Max	0.32	0.12	0.12
		Mean	0.03	0.03	0.02
		Median	0.02	0.02	0.02
		SE	0.01	0.00	0.00
	Adolescents (13 to 17.9 years)	Min	0.00	0.00	0.00
		Max	0.20	0.08	0.08
		Mean	0.02	0.02	0.01
		Median	0.01	0.01	0.01
		SE	0.01	0.00	0.00
	Adults (18 to 64.9 years)	Min	0.03	0.04	0.02
		Max	2.29	0.89	0.91
		Mean	0.24	0.21	0.17
		Median	0.14	0.13	0.11
		SE	0.07	0.03	0.02
Pb	Children (3 to12.9 years)	Min	0.00	0.00	0.00
		Max	0.02	0.02	0.01
		Mean	0.01	0.01	0.00
		Median	0.00	0.00	0.01
		SE	0.00	0.00	0.00
	Adolescents (13 to 17.9 years)	Min	0.00	0.00	0.00
		Max	0.01	0.01	0.01
		Mean	0.00	0.00	0.00
		Median	0.00	0.00	0.00
		SE	0.00	0.00	0.00

	Adults (18 to 64.9 years)	Min	0.00	0.00	0.01
		Max	0.12	0.14	0.10
		Mean	0.05	0.04	0.03
		Median	0.03	0.03	0.04
		SE	0.01	0.00	0.00

Table 14 Values of hazard quotient of metal exposure from the consumption of hydroponically grown vegetables

Element	Population		Vegetable		
			Red coral	**Green oak**	**Coral lettuce**
As	Children (3 to12.9 years)	Min	0.01	0.00	0.01
		Max	0.04	0.03	0.04
		Mean	0.02	0.01	0.02
		Median	0.02	0.01	0.01
		SE	0.00	0.00	0.00
	Adolescents (13 to 17.9 years)	Min	0.00	0.00	0.01
		Max	0.03	0.02	0.03
		Mean	0.01	0.01	0.01
		Median	0.01	0.01	0.01
		SE	0.00	0.00	0.00
	Adults (18 to 64.9 years)	Min	0.04	0.02	0.07
		Max	0.30	0.20	0.29
		Mean	0.13	0.09	0.13
		Median	0.13	0.09	0.08
		SE	0.01	0.01	0.01
Cd	Children (3 to12.9 years)	Min	0.00	0.01	0.01
		Max	0.05	0.05	0.03
		Mean	0.02	0.02	0.01
		Median	0.01	0.02	0.01
		SE	0.00	0.00	0.00
	Adolescents (13 to 17.9 years)	Min	0.00	0.00	0.00
		Max	0.03	0.03	0.02
		Mean	0.01	0.01	0.01
		Median	0.01	0.01	0.01
		SE	0.00	0.00	0.00
	Adults (18 to 64.9 years)	Min	0.02	0.05	0.05
		Max	0.38	0.36	0.18
		Mean	0.11	0.15	0.10
		Median	0.10	0.14	0.10
		SE	0.01	0.01	0.00
	Children (3 to12.9 years)	Min	0.00	0.00	0.00
		Max	0.01	0.01	0.01
		Mean	0.00	0.01	0.00
		Median	0.00	0.01	0.00
		SE	0.00	0.00	0.00

Pb	Adolescents (13 to 17.9 years)	Min	0.00	0.00	0.00
		Max	0.01	0.01	0.01
		Mean	0.00	0.00	0.00
		Median	0.00	0.00	0.00
		SE	0.00	0.00	0.00
	Adults (18 to 64.9 years)	Min	0.01	0.00	0.01
		Max	0.08	0.08	0.06
		Mean	0.02	0.04	0.03
		Median	0.02	0.04	0.03
		SE	0.00	0.00	0.00

4.4.4 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 vegetables consumption. The summary of hazard index (HI) values is shown in Table 15.

The sum of the hazard indexes (HI) of three metals was higher than the permissible limit. 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 for the soil-grown vegetables are substantially greater ($p<0.05$) than hydroponic ones. The contribution of Cd to overall the 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 1.17 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 to population.

As a result of HI values greater than the acceptable, several health impacts can be developed after long term consumption of leafy vegetables. For example, the first sign of acute arsenic poisoning symptoms includes vomiting, diarrhea, and stomach pain, and long-term exposure can lead to serious health problems like skin cancer (Argos et al., 2010). The Cd poisoning can occur especially as a result of inhalation (e.g, fume of cadmium) or consumption of Cd contaminated foods. Cadmium has a biological half-life of 10-35 years in humans, with the kidneys being the primary site of accumulation (WHO, 2019). Diabetes, renal disease, and heart attacks have all been linked to chronic cadmium exposure. There is sufficient evidence to indicate their carcinogenicity in humans due to their extended and intensive occupational exposure to Cd (e.g., through Cd fume) (Obaid et al., 2012). Poisoning with Pb can harm the neurological system in both adults and children. Several studies have shown that Pb has negative impacts on both children and adults. High blood Pb levels are typically associated with poorer IQ, hearing acuity, speech, language deficits, growth delays, and signs of anti-social behavior in adolescents (World Health Organization, 2019). High Pb exposure has also been related to impacts on adult reproduction, including decreased sperm count in males and spontaneous abortion in women (Henry et al., 2007).

Table 15 The values of hazard index (HI) through consumption of different 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
(3 to 12.9	Cd	0.38	0.09	0.18	0.10	0.17	0.06
years)	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
(13 to 17.9	Cd	0.24	0.06	0.12	0.06	0.11	0.04
years)	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
(18 to 64.9	Cd	2.76	0.63	1.31	0.71	1.23	0.45
years)	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

4.5. Carcinogenic health risks from 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 Tables 16 and 17. Since As is the only element which is classified as a Class A cancer according to EPA's Integrated Risk Information System (IRIS) (US.EPA, 2011), 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 vegetable consumption in children, adolescents and adults ranged from 7.08×10^{-7}, 4.54×10^{-7} and 5.14×10^{-6}. In addition, the cancer risk of As exposure in the adult population through leafy vegetables consumption in this study was 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 16 Cancer risk of As exposure through soil grown leafy vegetables consumption

Element	**Population**		Vegetable		
			Red coral	Green oak	Coral lettuce
As	Children (3 to12.9 years)	Min	1.74×10^{-8}	2.83×10^{-8}	5.63×10^{-8}
		Max	5.11×10^{-7}	2.18×10^{-7}	7.08×10^{-7}
		Mean	1.67×10^{-7}	1.03×10^{-7}	1.65×10^{-7}
		Median	1.16×10^{-7}	9.77×10^{-8}	1.30×10^{-7}
		SE	1.96×10^{-7}	5.99×10^{-9}	1.77×10^{-8}
	Adolescents (13 to 17.9 years)	Min	1.12×10^{-8}	1.82×10^{-8}	3.61×10^{-8}
		Max	3.28×10^{-7}	1.40×10^{-7}	4.54×10^{-7}
		Mean	1.07×10^{-7}	6.62×10^{-8}	1.06×10^{-7}
		Median	7.42×10^{-8}	6.27×10^{-9}	8.33×10^{-8}
		SE	1.26×10^{-8}	3.84×10^{-9}	1.13×10^{-8}
	Adults (18 to 64.9 years)	Min	1.26×10^{-7}	2.05×10^{-7}	4.09×10^{-7}
		Max	3.71×10^{-6}	1.58×10^{-6}	5.14×10^{-6}
		Mean	1.21×10^{-6}	7.49×10^{-7}	1.20×10^{-6}
		Median	8.40×10^{-7}	7.09×10^{-7}	9.42×10^{-7}
		SE	1.42×10^{-7}	4.35×10^{-8}	1.28×10^{-7}

Table 17 **C**ancer risk of As exposure through consumption of hydroponically grown vegetables

Element	Population		Vegetable		
			Red coral	Green oak	Coral lettuce
As	Children (3 to12.9 years)	Min	3.54×10^{-8}	1.47×10^{-8}	6.12×10^{-8}
		Max	2.69×10^{-7}	1.74×10^{-7}	2.57×10^{-7}
		Mean	1.13×10^{-7}	8.00×10^{-8}	1.14×10^{-7}
		Median	1.17×10^{-7}	8.08×10^{-8}	7.35×10^{-8}
		SE	8.43×10^{-9}	4.63×10^{-9}	1.12×10^{-8}
	Adolescents (13 to 17.9 years)	Min	2.27×10^{-8}	9.43×10^{-9}	3.93×10^{-8}
		Max	1.73×10^{-7}	1.11×10^{-7}	1.65×10^{-7}
		Mean	7.25×10^{-8}	5.13×10^{-8}	7.31×10^{-8}
		Median	7.49×10^{-8}	5.18×10^{-8}	4.71×10^{-8}
		SE	5.41×10^{-9}	2.97×10^{-9}	7.16×10^{-9}
	Adults (18 to 64.9 years)	Min	2.57×10^{-7}	1.07×10^{-7}	4.44×10^{-7}
		Max	1.96×10^{-6}	1.26×10^{-6}	1.86×10^{-6}
		Mean	8.20×10^{-7}	5.81×10^{-7}	8.28×10^{-7}
		Median	8.47×10^{-7}	5.87×10^{-7}	5.33×10^{-7}
		SE	6.12×10^{-8}	3.36×10^{-8}	8.10×10^{-8}

Remark: CR is cancer risk

CHAPTER V
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study observed the concentrations of As, Cd, and Pb in different leafy salad vegetables that were sold in Bangkok's fresh markets at 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 leafy vegetable consumption. The key results and findings from this study are listed as follow.

1. The concentrations of all metal of interests in the leafy vegetables grown in soil were always higher than those contained in the hydroponic leafy vegetables.
2. The total As concentration of leafy vegetables was in the order of red coral > green oak > coral lettuce. The maximum As concentration was found in soil grown red coral.
3. The total Cd concentrations in all leafy vegetables were higher than the MOPH standard. Interestingly, red coral had the highest concentration among the three vegetables for both cultivations. About 25% of all vegetable samples contained Cd and lettuce consumption was not safe for Cd exposure.
4. The total Pb concentrations in all vegetables were lower than those recommended by MOPH. The highest levels were found in green oak grown in soil, followed by red coral and coral lettuce.

5. The hazard quotient (HQ) of Cd exposure was greater than the HQ values for As and Pb, HQ levels of Cd in this study indicated potential negative non-carcinogenic effects in population.
6. The hazard index (HI) values of metals through consumption of leafy vegetables were predominantly dominated by Cd exposure.
7. Adults are the group of population who may encounter non-carcinogenic health impacts more than those of adolescents and children.
8. For carcinogenic health risk, the cancer risk of As exposure in the population through leafy vegetables 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 this research finding, regular heavy metal monitoring in agricultural regions is required to identify and prevent an excessive buildup of these metals in the human food chain. Based on 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. Thus, public awareness of the public health implications, particularly the non-carcinogenic health effects of metal contamination, should be enhanced to assure their safety via vegetable consumption. Moreover, practical, and general recommendations should be introduced to reduce the metal's exposure via lettuce consumption.

5.2 Recommendations

The following recommendations should be further studied in order to obtain more accurate risk information and a better understanding of toxic metals contamination in vegetables.

1. To assure that the samples are representative, the sample size should be increased for each type of vegetable studied.
2. To cover the entire country's population, the study area should be expanded.
3. The samples should be taken from the farms to study the actual effects cultivation methods and environmental factors affecting the levels of metals accumulation in vegetables.

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