

Determination of toxic heavy metal contaminated in food crops in Nakhon Pathom province, Thailand

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ABSTRACT: The contamination of toxic heavy metals including mercury (Hg), arsenic (As), cadmium (Cd), and lead (Pb) in food crops collected from Nakhon Pathom province was investigated. Fourteen crop species (n=42) were obtained from three public markets, namely, Sampran, Muang, and Phutthamonthon. Mercury was analyzed by Cold Vapor-Atomic Absorption Spectrophotometer (CV-AAS) while As, Cd, and Pb were simultaneously determined by Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). Results indicated that the ranges of Hg, Pb, Cd, and As contents in crop samples for fresh weight were 0.005-2.468, 0.001-0.094, 0.001-0.028, and 0.001-0.156 mg kg⁻¹, respectively. The heavy metal residue levels were ranked as follows: Cd < Pb < As < Hg. The Pb and Cd contents in all analyzed samples were below the permissible limits of Pb established by the Ministry of Public Health in Thailand and the permissible limits of Cd established by Codex; FAO/WHO. However, 78.57% of analyzed samples including pak choi, lettuce, and chinese cabbage showed that the levels of Hg exceeded the permissible limits (0.02 mg kg⁻¹) set by the Ministry of Public Health, Thailand. 35.71% of analyzed samples such as basil, chinese cabbage, kangkung, lettuce, rice, and spring onion showed that the levels of As exceeded the permissible limit (0.02 mg kg⁻¹) of As from Codex; FAO/WHO. These results represented information of contaminated toxic heavy metals in crop samples for the food safety in this area. Additionally, the sources of heavy metal contamination were discussed in this study.

Keywords: Heavy metal, Food crops, Cold Vapor-Atomic Absorption Spectroscopy, Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), Nakhon Pathom

Introduction

The main part of food crops such as vegetables, rice, cereals, and spices provide vitamins, fiber, and minerals to support human health and decrease the risk of chronic diseases (Broekmans et al., 2000). However, the consumption of food crops is the main source that the human body can accumulate both essential trace elements and toxic heavy metals. Currently, the contamination of toxic

heavy metals, e.g., mercury (Hg), lead (Pb), cadmium (Cd), and arsenic (As) in food crops exceeded the guideline of the European Union (Mahmood and Malik, 2014) and WHO/FAO (Ghasemidehkordi et al., 2018) were found. The high level of these heavy metals was reported as the cause of acute and chronic diseases as shown in Table1. Hence, Hg, Pb, Cd, and As are classified as the most toxic heavy metals due to their hazardous nature

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Table 1 Potential sources and toxicity of Hg, As, Cd, and Pb.

Heavy metals	Potential sources	Toxicity
Mercury (Hg) (Gibb and O'Leary, 2014)	<ul style="list-style-type: none"> - Processing of batteries, lamps, and dental fillings - Using of pesticides and fungicides 	<ul style="list-style-type: none"> - Damages nervous system, digestive system, and immune system - Toxic to lungs, kidneys, skin, and eyes
Lead (Pb) (WHO, 2010c)	<ul style="list-style-type: none"> - Mining, smelting, fossil fuels, and processing of batteries, paint, and soldering material 	<ul style="list-style-type: none"> - Chronic damage in gastrointestinal system, neurologic system, hematologic system, cardiovascular system, and renal system
Cadmium (Cd) (WHO, 2010b)	<ul style="list-style-type: none"> - Processing of batteries, pigments, metal coatings, and plastics 	<ul style="list-style-type: none"> - Induce carcinogenesis in skeletal system and respiration system - Damages lungs and kidneys
Arsenic (As) (WHO, 2010a)	<ul style="list-style-type: none"> - Processing of glass, pigments, paper, textiles, and smelters - Using of pesticides and phosphate fertilizers 	<ul style="list-style-type: none"> - Induce carcinogenesis in skin, lungs, liver, and bladder - Toxic to nervous system and pulmonary system - Cause cardiovascular disease

and are considered the top ten hazardous chemicals by the World Health Organization (WHO) (WHO, 1980).

In developing countries, the causes of toxic heavy metal contamination in agricultural products and the environment were by human activities, for example, mining (Luo et al., 2014), smelting, electronic waste (Damrongtsiri et al., 2016), and industrial factories (Huang et al., 2014; Bi et al., 2018). Additionally, agricultural activities including pesticides, fungicides, and fertilizers were reported as the major source of heavy metals in vegetable farms from China (Ning et al., 2017). Cd, Co, Cu, and Zn were the composition of fertilizers. Fe, Mg, Zn, Pb, and Ni were used for herbicides (Gimeno-García et al., 1996), and Hg and As were found in fungicides (Turull et al., 2018). These heavy metals can be easily absorbed by plants, leading to accumulation

on crops. For instance, the accumulation of Hg was found in plant tissue and its accumulated level increased in the order of grain, stalks, roots, and leaves, respectively (Feng et al., 2006). Certain vegetables (Zhuang et al., 2016), plants (Siriangkhawut et al., 2017), and food crops (Van Geen et al., 2006) were reported as the risk factor for the accumulation of heavy metals in humans. Thus, the residue of toxic heavy metals in crops can cause serious human health problems.

Thailand is an agricultural country that is located in Southeast Asia. This land provides agricultural produce to serve human consumption. However, the content of the toxic heavy metals in agricultural produce from certain areas was found to be above the permissible value from the Ministry of Public Health in Thailand (Meepun et al., 2014; Wachirawongsakorn, 2016). Nakhon

Pathom is the one of the central provinces of Thailand that covers an area of 2,168.327 km² with 58.75% being the agricultural area. There are seven districts, namely, Mueang Nakhon Pathom, Kamphaeng Saen, Nakhon Chai Si, Don Tum, Bang Len, Sam Phran, and Phutthamonthon. The population of this area is about 900,000. Most of the land consists of plains along a river which is fertile and suitable for agriculture. The main food crops in this province are baby corn, yard-long bean, cabbage, lettuce, spring onion, basil, kale, Chinese cabbage, fingerroot, pak choi, chili, kangkung, cucumber, and rice. There were 522,631,000 kg of food crops which were produced from this land in 2017 (Nakhon Pathom Provincial Office, 2018). Although, Nakhon Pathom is the land of food production, there are more than 3,000 industrial factories in this area including leather, textiles, petroleum, metals processing, and fertilizer. Hence, these activities can cause a risk of heavy metal contamination in the environment and the food chain. However, there is no research that studies the contamination of toxic heavy metal in food crops from Nakhon Pathom.

Herein, the contamination of toxic heavy metals including of Hg, As, Cd, and Pb in food crops from 3 public markets in Nakhon Pathom province, Thailand were investigated. The levels of heavy metal were measured using Cold Vapor-Atomic Absorption Spectrophotometer (CV-AAS) and Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). The values of heavy metals in crop samples were compared to the values of the quality standards of the Ministry of Public Health, Thailand and Codex Alimentarius guidelines by FAO/WHO. These findings can provide effective information on heavy metals

contamination food crop products in Nakhon Pathom in the future.

Materials and Methods

Chemical and reagents

All chemicals and reagents were analytical grade. Hydrochloric acid, nitric acid, and hydrogen peroxide were purchased from Merck (Darmstadt, Germany). All stock solutions were prepared by Milli-Q water. Nitric acid and hydrogen peroxide were used for digestion of the dried samples. Stannous chloride and hydrochloric acid were the reducing agent for mercury analysis. Mercury standard at the concentration of 1000 µg mL⁻¹ was purchased from SCP Science. Multi-element calibration standard (contained 10 mg mL⁻¹ of Ag, Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Ti, U, V, and Zn) and internal calibration standard solutions (contained 10 mg mL⁻¹ of Bi, Ge, In, Li, Sc, Tb, and Y) were purchased from Perkin Elmer Pure Plus. Multi-element calibration standard solution was used to prepare the calibration curve of Pb, As, and Cd. Internal calibration standard solution was applied for quality control analysis of Pb, As, and Cd. All standards and samples solutions were diluted with 1 % (v/v) of nitric acid solution in Milli-Q water before analysis.

Food crop sampling

Fourteen species of food crops, namely, baby corn (*Zea mays* Linn.), yard-long bean (*Vigna unguiculata*), cabbage (*Brassica oleracea* var. *capitata* L.), lettuce (*Lactuca sativa*), spring onion (*Allium cepa* var. *aggregatum*), basil (*Ocimum basilicum*

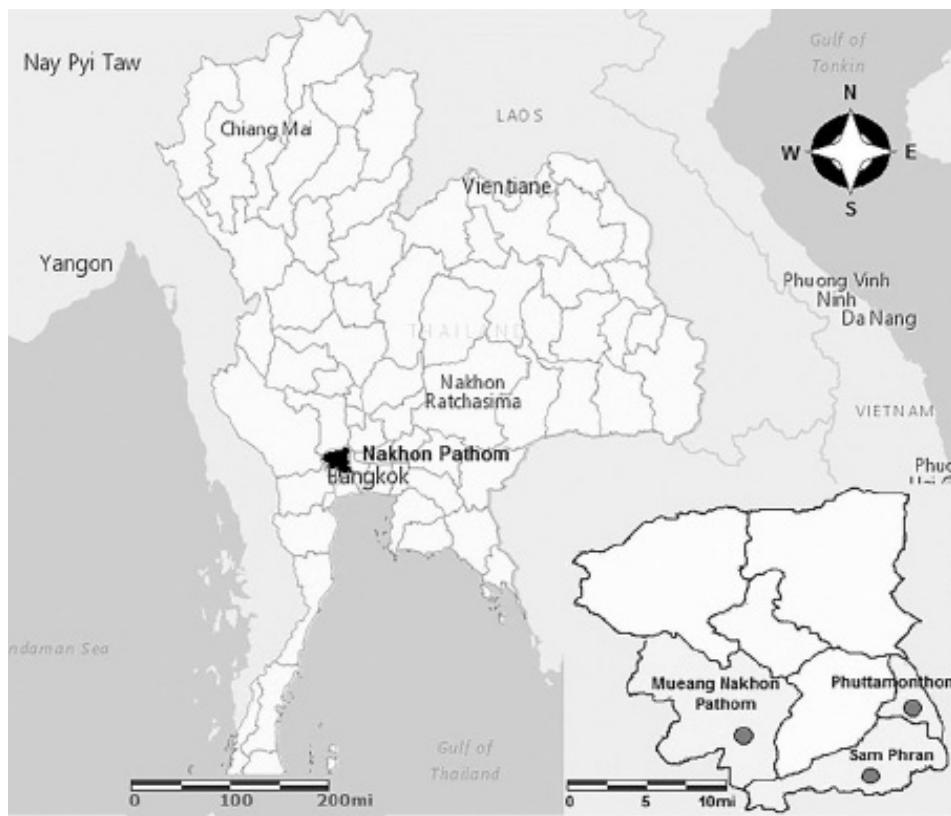


Figure 1 Location of three public market sites in the Nakhon Pathom province, Thailand.

Linn.), kale (*Brassica alboglabra*.), Chinese cabbage (*Brassica pekinensis*.), fingerroot (*Boesenbergia rotunda*), pak choi (*Brassica chinensis* *Jusl* var *parachinensis* (*Bailey*) *Tsen* & *Lee*.), chili (*Capsicum frutescens* *Linn.*), kangkung (*Ipomoea aquatica* *Forsk.*), cucumber (*Cucumis Sativus* *Linn.*), and rice (*Oryza sativa* *L.*) were purchased from 3 public markets in Nakhon Pathom province, namely, Sampran, Mueang Nakhon Pathom, and Phutthamonthon districts (see in Figure1). In this work, the three markets were concealed by using the pseudonym which was market A, market B, and market C. All crop samples were kept in clean zip lock polythene bags and transported to the laboratory. The fresh

samples were washed then air-dried at room temperature. After that, they were cut into small pieces. All samples were dried in a hot air oven at 80 °C for 24 hours (Islam and Hoque, 2014), excepting for the finger root that was dried for 48 hours. The samples were weighed before and after drying. Then the dried samples were powdered by mortar and collected in polythene screw cap tubes. Finally, the samples were stored at 4 °C until required for digestion.

Sample digestion

All vessels and containers were soaked in 10% HNO_3 for 24 hours and rinsed with Milli-Q water and air-dried before use. For each experiment, 0.5 grams of powdered

samples were weighed and added to cleaned vessels. Then, digestion was performed by adding 6 mL of 65% HNO_3 and 2 mL of 30% H_2O_2 to the vessel according to digestion protocol of Microwave digestion (MLS-1200 MEGA of MILESTONE). Next, the vessel was cooled at room temperature and the digested sample was transferred into a polythene screw cap tube. After that, 30 mL of Milli-Q water was added to the digested sample. Finally, the digested sample was analyzed by Cold Vapor-Atomic Absorption Spectrophotometry (CV-AAS) for Hg and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) for Pb, Cd, and As.

Hg analysis

The concentration of mercury in samples was determined by using the Cold Vapor-Atomic Absorption Spectrophotometer (PerkinElmer Flow Injection Mercury Systems 100 and 400; FIMS series). 500 μL of the sample solution was prepared in a mixture of 1.1% (v/v) SnCl_2 and 3% (v/v) HCl and pumped through the reactor with the aid of the peristaltic pump. Then, the elemental mercury vapor was generated and entered to the quartz cell for analysis of mercury. The standard curve for Hg analysis was established by using standard Hg solution (5, 10, and 20 $\mu\text{g L}^{-1}$). The internal quality control was done for every 10 samples by using the standard Hg solution at the concentration of 2 $\mu\text{g L}^{-1}$ and 15 $\mu\text{g L}^{-1}$ to make sure that the CV-AAS system is working accurately. The percentage of relative standard deviation (%RSD) value was controlled in the range of 10% to verify the precision. The signal was measured at a maximum wavelength of 253.7 nm, then the absorbance value was converted as the concentration of Hg in the

unit was $\mu\text{g L}^{-1}$.

Pb, Cd, and As analysis

The concentration of Pb, Cd, and As were determined by using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS, PerkinElmer NexION300XSeries). The standard curve was established by using a multi-element standard solution at a concentration of 25, 50, 75, and 100 $\mu\text{g L}^{-1}$. The internal calibration standard solution mix (PerkinElmer) contained 10 mg L^{-1} of bismuth (Bi), germanium (Ge), indium (In), scandium (Sc), lithium (Li), terbium (Tb), and yttrium (Y). Standard, internal standard, and digested sample solutions were prepared in 1% (v/v) of HNO_3 . The internal quality control was performed to obtain the accuracy and precision for every 10 samples. The measurement of each analyzed samples was duplicated ($n=2$). The optimized conditions for Pb, Cd, and As analysis were 1.05 L min^{-1} of nebulizer gas flow, 1.35 L min^{-1} of auxiliary gas flow, 18 L min^{-1} of plasma gas flow, and 1600 W of radio-frequency (RF) in an ICP system.

Results and Discussion

The toxic heavy metals of Hg, Pb, Cd, and As can cause serious health problems (Table 1). Hg can damage the nervous, digestive, and immune systems while Pb can damage gastrointestinal, neurologic, hematologic, cardiovascular, and renal systems. The accumulation of Cd in the human body for long periods can induce carcinogenesis in skeletal and respiration systems and damage lungs and kidneys. The exposure to As can induce carcinogenesis in skin, lungs, liver, and bladder, and is toxic to the nervous system and pulmonary system.

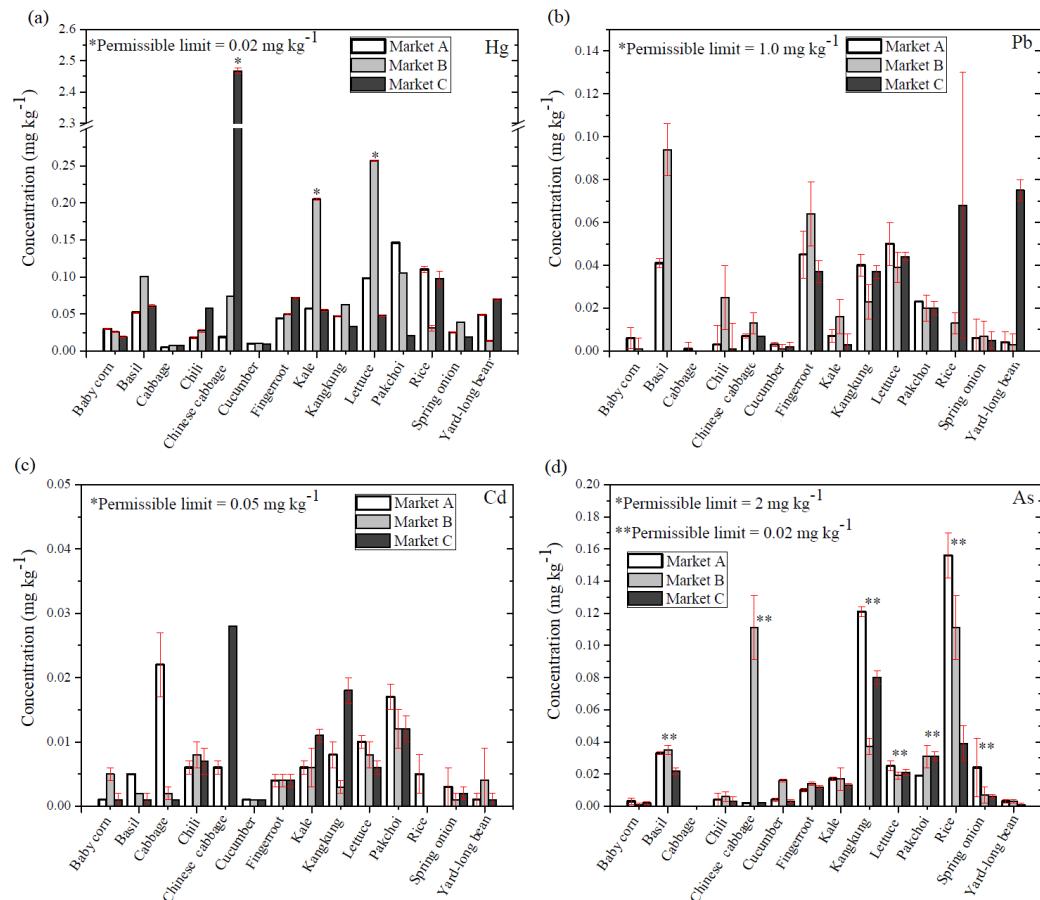


Figure 2 The mean concentration of (a) Hg, (b) Pb, (c) Cd, and (d) As in the 14 species of fresh crops collected from three markets in Nakhon Pathom (mg kg^{-1} , fresh weight). The vertical line represents the standard deviation (SD). (*) and (**) show the heavy metal content exceeded the permissible limit from the Ministry of Public Health in Thailand and Codex Alimentarius guidelines, respectively.)

Therefore, in this work, the accumulation of Hg, Pb, Cd, and As in food crop samples (14 species) from Nakhon Pathom area were studied. Moreover, the mean concentration of heavy metals founded in samples was presented in Figure 2. These heavy metal contents were compared with the permissible limit from the Ministry of Public Health in Thailand and Codex Alimentarius guidelines

by FAO/WHO.

Hg in food crop samples

Hg is a toxic heavy metal that can be taken into the human body via the respiratory system, gastrointestinal tract, and skin. Although several work has reported the toxicity of Hg for human health and contamination in the environment, the accumulation of Hg in food crops in Nakhon Pathom province were

not investigated. In this study, the Hg content in collected crop samples was analyzed by CV-AAS technique. The lowest and highest measurements of Hg content in the collected crops from market A were found in cabbage (0.005 mg kg^{-1}) and pak choi (0.146 mg kg^{-1}), respectively. In market B, the lowest and highest measurements of Hg content in the samples were reported in cucumber (0.010 mg kg^{-1}) and lettuce (0.257 mg kg^{-1}), respectively. In market C, cucumber (0.009 mg kg^{-1}) and Chinese cabbage (2.468 mg kg^{-1}) were reported as having the lowest and highest Hg content, respectively. The status of Hg contamination in food crops was compared with the permissible limit from the Ministry of Public Health in Thailand (Ministry of Public Health in Thailand, 2017). This permissible limit is 0.02 mg kg^{-1} in a fresh weight sample. The Hg content was detected in 100% of all analyzed samples and 78.57% of analyzed samples exceeded this permissible limit, except cucumber and cabbage. Moreover, the mean concentration of Hg for all fresh crops was presented in Figure 2a. The mean concentration varied in the range of 0.01 - 0.85 mg kg^{-1} and decreased in the following order: Chinese cabbage, lettuce, kale, pak choi, rice, basil, fingerroot, kangkung, yard-long bean, chili, spring onion, baby corn, cucumber, and cabbage, respectively. It means that a high level of Hg was found in leaf crops such as Chinese cabbage, lettuce, kale, etc. This result agreed with the previous study that the level of Hg in plant tissue increased in the order of grain, stalk, root, and leaves, respectively (Zheng et al., 2007). Additionally, B.A. Zarcinas and coworkers found that the highest Hg content in rice (0.022 mg kg^{-1}) which was

randomly collected from the northeast region of Thailand exceeded the permissible limit (0.02 mg kg^{-1}), whereas the Hg content in soil which was collected from this growing area did not exceed the permissible limit of Hg in soil (0.3 mg kg^{-1}) (Zarcinas et al., 2004). Therefore, the contamination source of heavy metal in this work may have occurred from agricultural activity, because heavy metals are an essential composition of fungicide. For example, methoxy ethyl mercuric chloride (MEMC) have been wildly used as a fungicide to protect root and seeds of food crops (Turull et al., 2018).

Pb in food crop samples

Pb is one of the toxic heavy metals widely used in mining and industrial activities. Moreover, fertilizers such as copper sulfate and iron sulfate present significant content of Pb (Gimeno-García et al., 1996). The contamination of Pb was widely found in the environment and food. In this study, Pb was investigated in crop samples from three fresh markets by using ICP-MS analysis. The lowest and highest content of Pb in samples from market A was found in rice ($< 0.001 \text{ mg kg}^{-1}$) and lettuce (0.050 mg kg^{-1}), respectively. In the case of market B, the lowest and highest content of Pb in samples was found in cabbage ($< 0.001 \text{ mg kg}^{-1}$) and basil (0.094 mg kg^{-1}), respectively. In market C, the lowest Pb content was reported in baby corn, basil, and cabbage ($< 0.001 \text{ mg kg}^{-1}$), while the highest Pb content was reported in yard-long beans (0.075 mg kg^{-1}). Figure 2(b) presents the mean concentration of Pb in crop samples which varied in the range of 0.0003 - $0.0487 \text{ mg kg}^{-1}$ and increased in the following order: cabbage, cucumber, baby corn, spring onion,

kale, Chinese cabbage, chili, pak choi, rice, yard-long bean, kangkung, lettuce, basil, and fingerroot, respectively. Based on the current results, the average Pb content from samples in Nakhon Pathom was $0.0203 \pm 0.0263 \text{ mg kg}^{-1}$. However, the Pb content in all analyzed samples did not exceed the permissible limit from the Thai ministry of public health (1 mg kg^{-1}). This result corresponds to another study about the contamination of Pb in crops from the southern part of Thailand, which found that the average Pb content in crops did not exceed the permissible limit (Meepun et al., 2014). Moreover, most of the Pb content did not exceed the maximum permissible limit from Codex Alimentarius guidelines by WHO/FAO (0.1 mg kg^{-1}) (FAO-WHO, 2015). This result indicates that food crops in this area are safe from Pb accumulation.

Cd in food crop samples

Cd is one of the toxic heavy metals which is mainly used in the environment by batteries, pigment, metal coating, and plastic production. Moreover, the contamination of Cd in agricultural products was found in crop plants especially vegetables and rice which were reported as the highest contributors of Cd exposure in the population (Yang et al., 2017). The studies of contaminated Cd in food from polluted areas such as mining zones in the western region of Thailand were reported. However, there was a few reports about the exposure of Cd in food crops from the center region of Thailand (Chunhabundit, 2016). This is the first work that investigated Cd contamination in economical crops in the Nakhon Pathom area. The Cd levels were measured by the ICP-MS. In the case of market A, the lowest Cd content in crop samples was

reported in baby corn, cucumber, and yard-long bean (0.001 mg kg^{-1}), while the highest Cd content was reported in cabbage (0.022 mg kg^{-1}). For market B, the lowest Cd content in samples was found in Chinese cabbage and rice ($<0.001 \text{ mg kg}^{-1}$) while the highest Cd content in samples was found in pak choi (0.012 mg kg^{-1}). The lowest content of Cd in market C was found in rice ($<0.001 \text{ mg kg}^{-1}$), while the highest content of Cd was found in Chinese cabbage (0.028 mg kg^{-1}). On the other hand, the accumulation of Cd in crop samples was compared with another area of Thailand. Most selected crops samples from this province presented the lower Cd content when compared with the local vegetables from Surat Thani province (0.53 mg kg^{-1}) (Meepun et al., 2014). The mean concentration of Cd content in crop samples is illustrated in Figure 2c. All mean concentrations of Cd were below the maximum permissible limit from the Codex Alimentarius guidelines by WHO/FAO (0.05 mg kg^{-1}) (FAO-WHO, 2015). Similarly, Cd content in food crops from Bangkok did not exceed the maximum permissible limit as reported in previous work (Chunhabundit, 2016).

As in food crop samples

The toxic heavy metal As generally accumulates in food crops, particularly rice. The major sources of As were semiconductors, glass, pigments, paper, textiles, smelt manufacturing, pesticides, and phosphate fertilizers. The intake of As in food crops is a major pathway for As exposure to humans. In this work, the cabbage sample presented is the lowest As content for all sample sites ($<0.001 \text{ mg kg}^{-1}$). The highest As content in real samples from market A, B, and C were reported in rice (0.156 mg kg^{-1}),

Chinese cabbage and rice (0.111 mg kg^{-1}), and kangkung (0.080 mg kg^{-1}), respectively. **Figure 2d** presents the mean concentration of As for fresh crop species from Nakhon Pathom. Content varied in the range of < 0.001 - 0.102 mg kg^{-1} and increased in the following order: cabbage, Chinese cabbage, baby corn, yard-long bean, chili, cucumber, fingerroot, spring onion, kale, lettuce, pak choi, basil, kangkung, and rice, respectively. The Ministry of Public Health in Thailand established the maximum permissible content of As in food crops at 2 mg kg^{-1} . Therefore, the As content in all samples was below the maximum permissible value. However, 35.71% of analyzed samples such as basil, Chinese cabbage, kangkung, lettuce, rice, and spring onions exceeded the permissible limit from Codex; FAO/WHO, 2017 for As (0.02 mg kg^{-1}). Rice was reported as the highest As accumulated plant when compared to another crop (van Geen et al., 2006). Importantly, the high level of As in soil was found as one factor for As accumulation in cabbage, corn, and rice from Thailand (Zarcinas et al., 2004).

From the results of heavy metal contamination in crops, the order of mean concentration of heavy metals was $\text{Hg} > \text{As} > \text{Pb} > \text{Cd}$. The highest content of Hg, As, Pb, and Cd was observed in Chinese cabbage, fingerroot, pak choi, and rice, respectively. Cd and Pb content in all collected crop samples from public markets did not exceed the permissible limit of the Thai Ministry of Public Health and Codex; FAO/WHO, 2017 guideline. However, Hg and As content in crop samples exceeded this threshold. Based on the previous study, the cause of Hg and As contamination

was unclear and it was dependent on several sources such as fertilizers, pesticides, fungicides, and industrial effluent. From this finding, the sources of heavy metals should be studied in near future in order to prevent the food contamination.

Conclusions

The contaminated toxic heavy metals including of Hg, As, Cd, and Pb in crop samples in Nakhon Pathom province was investigated by Cold Vapor-Atomic Absorption Spectrophotometry (CV-AAS) and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) for Hg and Pb, Cd, and As, respectively. The content of As, Cd, and Pb in crop samples did not exceed the maximum permissible limit from the Ministry of Public Health in Thailand. However, exceeded Hg content in samples was found in baby corn, basil, chili, Chinese cabbage, fingerroot, kale, kangkung, lettuce, pak choi, rice, spring onion, and yard-long bean. It is suggested that finding the cause of toxic metal contamination in crop fields may be necessary for food safety in human health especially in the Nakhon Pathom province.

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