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Polycyclic Aromatic Hydrocarbons in Thai and Myanmar Rice: Concentrations, Distribution and Health Concerns

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ABSTRACT

In the present study, we studied the concentrations and proportions. We identified the potential sources and health risks of 12 probably carcinogenic polycyclic aromatic hydrocarbons (PAHs) in rice grain from 31 sites in Thailand and Myanmar. The findings showed that PAH concentrations in rice grain samples from Thailand and Myanmar were in the range of 0.09 to 37.15 $ng.g^{-1}$ with an average value of 18.22 ± 11.76 $ng.g^{-1}$ and 0.07 to 150.73 $ng.g^{-1}$ with an average value of 34.70 ± 40.57 $ng.g^{-1}$, respectively. The majority group of PAHs in the rice grain samples from Thailand were the five-ring PAHs (78%), followed by four-rings (12%) and three-ring PAHs (9.5%), respectively, while for Myanmar was the five-ring PAHs were the majority (64.02%), followed by six-rings (15.22%) and four-ring PAHs (13.58%), respectively. The diagnostic ratio analysis suggested that pyrogenic origin is a major source of PAHs, and principal component analysis (PCA) identifies the incomplete combustion of fuel as likely the primary source of emissions source of PAHs contamination in rice grain samples. The total values of incremental lifetime cancer risk (ILCR) of PAH content of rice grain for children and adults were 1.95×10^{-8} and 1.44×10^{-8}, respectively, for Thailand and 1.83×10^{-7} and 1.35×10^{-7} for Myanmar, which showed that the incremental lifetime cancer risk from rice grain was lower than the baseline set is considered to be safe levels.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) contain two or more benzene rings and are classified as persistent organic pollutants (Deelaman et al.2020a, 2021). It is generally recognized that both natural and anthropogenic combustion plays a major role in the formation of PAHs (Bamforth & Singleton 2005, Ravindra et al. 2008, Zhang & Tao 2009, Abdel-Shafy & Mansour 2016). Since many PAHs have been released into the environment due to swift industrial and economic development, various governments are currently concentrating on controlling PAHs. Some PAHs originate from the incomplete combustion of fuels, such as oil, gas, coal, and wood, for energy supplies, including vehicles and machinery (Maliszewska-Kordybach 1999, Qin et al. 2018, Zhu et al. 2019). Moreover, PAHs are released while manufacturing dyes, plastics, agrochemicals, resins, and pesticides (Abdel-Shafy & Mansour 2016). According to the World Health Organization, around 50% of people in Africa and 75% in Southeast Asia, India, and China use wood-based fuels for daily cooking. More than 90% of the concentration of PAHs in the environment is estimated to be attributable to combustion sources (Neilson 1998).

PAHs can be found in several components of the environment, such as the atmosphere, soil, water, sediment, vegetables, food, and adipose tissue of living organisms (Bamforth & Singleton 2005, Pongpiachan et al. 2017, ChooChuay et al. 2020, Deelaman et al. 2020b). The International Agency for Research on Cancer under the World Health Organization has designated that 17 types of PAHs are of the greatest concern with adverse health effects on humans, and the US Environmental Protection Agency (USEPA) has identified seven PAHs as probable human carcinogens (IARC 2010, Kim et al. 2013, Abdel-Shafy & Mansour 2016). The main issue with PAHs is that they can reactively metabolize; some can attach to DNA and proteins in cells, resulting in cell damage, tumor formation, and mutations. High PAH concentrations in the ecosystem can affect living organisms, such as fish, shellfish, and birds (Tudoran & Putz 2012, Inomata et al. 2012). Additionally, PAHs can enter the human body by eating, touching, and inhaling contaminated plants. Plants can absorb PAHs from polluted soil through their roots and transfer them to other parts (bio-accumulate) (Dong et al. 2012, Veltman et al. 2012, Abdel-Shafy & Mansour 2016).

Approximately half of the world's population consumes rice (*Oryza sativa* L.) as a staple food, particularly in Asia. By 2050, the world population is estimated to reach 9.8 billion (United Nations 2017, Liu et al. 2019, Fried et al. 2021). In the following decades, global population expansion is anticipated to increase demand for agricultural products (Foley et al. 2011, Alexandratos & Bruinsma 2012, Martin & Sauerborn 2013, Fried et al. 2021). Southeast Asia has a large agricultural area, accounting for 32% of the total land surface in 2018, with 50 million ha of rice plantation (Fried et al. 2021). In 2019, Thailand was the world's sixth producer of rice after China, India, Indonesia, Bangladesh, and Vietnam, with an estimated harvest of 28 million tons, which accounted for approximately 3.8% of the world's rice output (FAO 2020. Myanmar, which borders Thailand, is the seventh largest rice producer worldwide, harvesting approximately 26 million tons of rice. The consumption behaviors of people in both countries are similar in that they primarily rely on rice for their daily food intake. Over the past decade, numerous studies have examined the PAH contents that contaminate the environment. Several studies have assessed the levels of PAH contamination in food and the effects of pollution sources. Studies on PAH contents in ten major foods (cereals, nuts, fruit, meat, fish and shellfish, beverages, seasoning, pulse crops, vegetables, and eggs) in Korea detected PAHs in seven major food groups: cereals, nuts, fruit, meat, fish, beverages, and seasoning (Lee et al. 2018).

Similarly, phenanthrene, fluoranthene, and pyrene are the PAHs detected in raw fruits and vegetables. Numerous studies have revealed that the PAH concentrations in fruits and vegetables may vary considerably, depending on the area surrounding the plant, aromatic hydrocarbons, or the product itself (Paris et al. 2018). However, studies on PAH contamination in rice, a staple food in many Asian countries, are limited. Therefore, to provide more information on the safety of rice consumption, focusing on and supporting the studies on this major food group are necessary. This study randomly collected rice grain samples from different areas of the two countries (Thailand and Myanmar). Qualitative and quantitative analyses of 12 PAHs in the rice grain samples were carefully conducted to (i) assess the concentrations and proportions of PAHs in the areas of Thailand and Myanmar; (ii) identify possible sources of PAHs in rice grains using diagnostic binary ratios; (iii) assess the risk of exposure to PAHs using toxicity equivalent concentration (TEQ) equations; moreover, this is the first assessment of health risks associated with farmers and consumers in the areas of 12 dominant PAHs that have been found in rice grain samples in each age range. We also compared the impact of the age range of affected rice grain samples on the risk.

MATERIALS AND METHODS

Sampling Sites

Rice grain samples were randomly collected from 11 sites in Thailand: northern (Phayao, Lamphun, Phichit, and Tak provinces), eastern (Ubon Ratchathani, Yasothon, and Loei provinces), central (Nakhon Sawan and Suphan Buri provinces), and southern (Phatthalung and Nakhon Si Thammarat provinces). Samples were collected from 20 sites in Myanmar, including Nwar Kyoe Aing, Taung Ywar Naung, Patheingyi, Yae Htwet, Amarapura, Pan Chi, Hpa Ye Kyun, and Myauk Kaing. Fig. 1 shows a sampling point with a global positioning system used to accurately locate each point. The sampling duration was from December 2017 to February 2018 (*n* = 31).

Sample Collection and Preparation

All sampling sites were situated in the city of agricultural areas. Rice grain samples were collected from fields grown for four months. All precautions for rice sampling followed the "Crop Sampling Protocols for Micronutrient Analysis" to avoid contamination (Stangoulis & Sison 2008). First, the rice grains were manually threshed and wrapped in pre-cleaned aluminum foil. The samples were stored in labeled plastic bags to avoid the degradation of PAH caused by heat, gas, and ultraviolet radiation when transported to the laboratory. Samples were ground to homogenize, wrapped in pre-cleaned aluminum foil, packed in a plastic bag, and stored at -20 °C in a refrigerator before chemical extraction.

Chemicals and Standards

A concoction of twelve PAHs was formed according to the Norwegian Standard (NS 9815: S-4008-100-T) (phenanthrene [Phe], anthracene [An], fluoranthene [Fluo], pyrene [Pyr], Benz [a]anthracene [B[a]A], chrysene [Chry], benzo[b] fluoranthene [B[b]F], benzo[k]fluoranthene [B[k]F], benzo[a] pyrene [B[a]P], indeno[1,2,3-cd]pyrene [Ind], dibenz[a,h] anthracene [D[a,h]A], and benzo[g, h, i] perylene [B[g, h, i] P]) with each 100 μg.mL^{-1} in toluene (1×1 mL). Additionally, deuterated-perylene [d_{12}-Per] and deuterated-fluorene [d_{10}-Fl] were synthesized as a mixture of recovery standard (IS) PAHs at a concentration of 100 μg.mL^{-1} in xylene. All

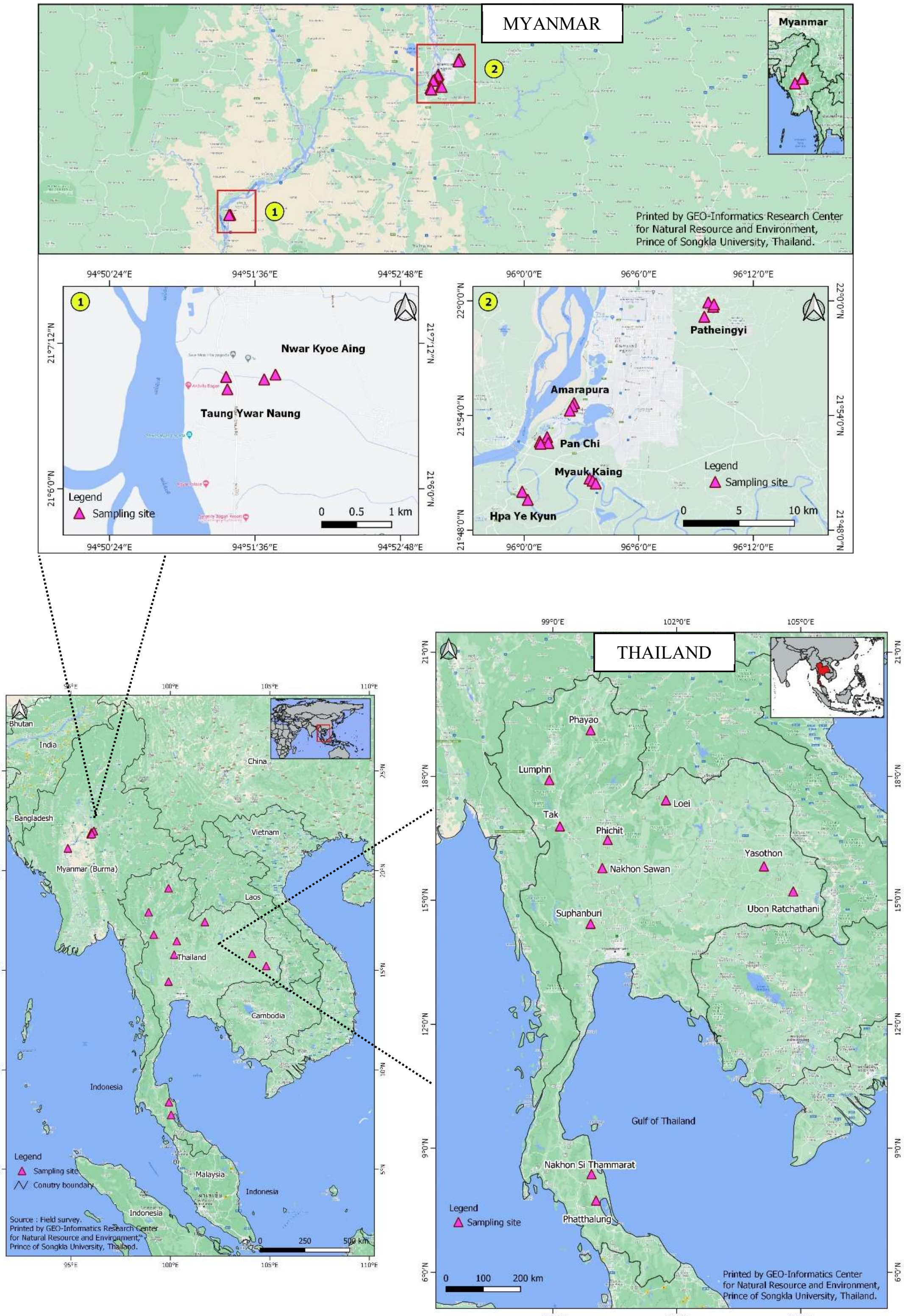

Fig. 1: Rice paddy sampling stations in Thailand and Myanmar.

substances utilized in this work were purchased from Chiron AS (Stiklestadveine 1, N-7041 Trondheim, Norway).

Sample Extraction, Cleanup and Analysis

This investigation used dichloromethane (DCM; Fisher Scientific) of high-performance liquid chromatography grade to chemically extract all rice grain samples, similarly to other organic solvents (e.g., hexane). A Soxhlet extraction thimble (cellulose fiber; size: 30 × 100 mm) was pre-cleaned and filled with around 50 g of the freeze-dried, homogenized rice grain sample. Subsequently, 50 μL was spiked with a known amount of internal reference standard before adding 250 mL of DCM to the sample along with 1 g of copper powder, and extraction was completed using a Soxhlet extractor for 8 h. Fractionation, cleanup, and blow-down were performed following the method described by Gogou et al. (1998). The pre-cleaned silica gel was then oven-dried at 150°C for 3 h, and the extract was transferred to the column. The PAH compound groups were classified by eluting with 15 mL of n-hexane and 5.6:9.4 (v/v) toluene: hexane. Subsequently, the solution was rotary evaporated and purged with a gentle nitrogen stream to almost dryness. The solvent was then changed to cyclohexane, and the volume was reduced with nitrogen again until the final volume was 100 μL; the solution was then quantified using a gas chromatograph/mass spectrometer (GC/MS; Shimadzu GCMS-QP, 2010 Ultra, Japan). Additional details about the GC/MS method can be found in previous studies (Pongpiachan et al. 2012, Deelaman et al. 2020a, b).

Source Identification of PAHs

Some PAHs are regarded as markers for specific environmental PAH-releasing processes. They are tracers, signatures, or origin markers (Ravindra et al. 2008). The relative thermodynamic stability of the various parent PAHs must be understood to use the PAH ratio for sediment attribution, according to Yunker et al. (2002). The relative stability of different PAH isomers and PAHs from different sources and the nature of different PAH sources can be used to determine their origin. The characteristics of the PAH patterns detected in the rice grains were consistent with the emission sources in the surrounding agricultural fields, where the PAH source can be distinguished based on the concentration and type of PAH found in the rice grain. PAHs with molecular weights of 178 and 202 were used to differentiate their sources between combustion and petroleum (Budzinski et al. 1997, Soclo et al. 2000, Yunker et al. 2002, Katsoyiannis & Breivik 2014). Diagnostic binary ratios of PAHs were employed to determine the sources of PAHs found in rice grains, which included six ratios of PAH isomer pairings. The details and PAHs emission rates for the various sources are shown in Table 1.

Table 1: Comparing the values obtained from this investigation with the characteristics of selected diagnostic ratios for petroleum, single-source combusation, and traffic emissions. (Modified from Yunker et al. 2002, Katsoyiannis & Breivik 2014)

Source	An/ (An+Phe)	Flou/ (Flou+Pyr)	B[a]A/ (B[a]A+ Chry)	Ind/ (Ind+ B[g,h,i]P)	B[a]P/ B[g,h,i]P	B[b]F/ B[k]F
Non-traffic emissions	-	-	-	-	< 0.6	
Traffic emissions	-	-	-	-	> 0.6	
Petroleum	< 0.10	< 0.40	< 0.20			
Crude oil (n=9) [a,b,c]	0.07	0.22	-	-	-	
Diesel oil (n=8) [d,e,f,g]	0.09	0.26	-	-	-	
Lubricating oil		0.29	0.10	0.12	-	
Asphalt[l,m]	-	-	0.50	0.52-0.54	-	
Combustion	> 0.10		> 0.35			
Bituminous coal (n=3)[h]	0.33	0.53	-	-	-	
Gasoline (n=2) [i,j]	0.11	0.44	0.33-0.38	0.09-0.22	-	0.73
Road dust	0.18	0.42	0.13	0.51	-	
Used engine oil, gasoline passenger car	0.22	0.30	0.50	0.18	-	
Used engine oil, diesel car, truck, and bus	-	0.37	-	0.29	-	> 0.50
Aluminum smelter emissions						2.50-2.90

[a] Grimmer et al. 1983. [b] Wise et al. 1988. [c] Benner et al. 1990.[d]Westerholm & Li 1994. [e] Wang et al. 1999. [f] Schauer et al. 1999. [g]Westerholm et al. 2001. [h] Oros & Simoneit 2000. [i] Li & Kamens 1993. [j] Rogge et al. 1993.

Toxicity Potential of PAHs Contamination of Rice Grains

Through dietary consumption, skin contact, and the respiratory system, PAHs may be bio-transferable and absorbed into human bodies, where they may lead to cancer. To estimate the risk of exposure to PAHs and the cumulative effects brought on by all potentially human carcinogenic PAHs, "B[a]P equivalents" were calculated using the toxic equivalency factors (TEFs) defined by the USEPA. The toxicity value estimated by B[a]P equivalent concentrations for Phe, Fluo, and Pyr was 0.001; for An, Chry, and B[g,h,i]P was 0.01; for B[a]A, B[b]F, B[k]F and Ind were 0.1, and for B[a]P and D[a,h]A were 1 (Nisbet & Lagoy 1992). The total TEQ can be calculated as follows:

$$\mathrm{TEQs} = \sum_{s} C_S \times \mathrm{TEF}_S \qquad \text{...(1)}$$

TEQs are the total toxicity equivalency concentrations, C_S are the concentrations of individual PAHs, and TEFs are toxic equivalency factors.

Health Risk Assessment of PAH Contamination Rice Grains

PAHs are generally found in the environment, exhibiting different types and chemical compositions. Each PAHs type has a specific toxicity level. Depending on the unique characteristics of PAHs, the bioaccumulation of PAHs in the adipose tissue of species varies significantly. Vegetables may be contaminated with PAHs by uptake from the soil through the roots and transportation to other parts. Additionally, plants can adsorb PAHs from gas-phase PAH deposition (Lu et al. 2014). The three main ways most people are exposed to PAHs in the environment are cutaneous absorption, inhalation, and ingestion (Ravindra et al. 2008, Deelaman et al. 2021). We calculated the risk of people exposed to PAHs in rice grains using the USEPA evaluation manual (USEPA 1991) by dividing the population into two groups: childhood and adulthood. Incremental lifetime cancer risk (ILCR) was used to evaluate the risks and potential health concerns associated with three different routes: inhalation ($\mathrm{ILCR}_{\mathrm{Inhalation}}$), dermal contact ($\mathrm{ILCR}_{\mathrm{Dermal}}$), and direct ingestion ($\mathrm{ILCR}_{\mathrm{Ingestion}}$). In this study, the rice grain ILCR can be calculated using the following equations 2, 3, 4, and 5, by the full details and variable description used according to Deelaman et al. (2021)

$$\mathrm{ILCR}_{\mathrm{Inhalation}} = \frac{\mathrm{CS}\times\left(\mathrm{CSF}_{\mathrm{Inhalation}}\times\sqrt[3]{\frac{\mathrm{BW}}{70}}\right)\times\mathrm{IR}_{\mathrm{inhalation}}\times\mathrm{EF}\times\mathrm{ED}}{\mathrm{BW}\times\mathrm{AT}\times\mathrm{PEF}} \qquad \text{...(2)}$$

$$\mathrm{ILCR}_{\mathrm{Dermal}} = \frac{\mathrm{CS}\times\left(\mathrm{CSF}_{\mathrm{Dermal}}\times\sqrt[3]{\frac{\mathrm{BW}}{70}}\right)\times\mathrm{SA}\times\mathrm{AF}\times\mathrm{ABS}\times\mathrm{EF}\times\mathrm{ED}}{\mathrm{BW}\times\mathrm{AT}\times 10^{6}} \qquad \text{... (3)}$$

$$\mathrm{ILCR}_{\mathrm{Ingestion}} = \frac{\mathrm{CS}\times\left(\mathrm{CSF}_{\mathrm{ingestion}}\times\sqrt[3]{\frac{\mathrm{BW}}{70}}\right)\times\mathrm{IR}_{\mathrm{ingestion}}\times\mathrm{EF}\times\mathrm{ED}}{\mathrm{BW}\times\mathrm{AT}\times 10^{6}} \qquad \text{...(4)}$$

$$\mathrm{ILCRs} = \mathrm{ILCR}_{\mathrm{Inhalation}} + \mathrm{ILCR}_{\mathrm{Dermal}} + \mathrm{ILCR}_{\mathrm{Ingestion}} \qquad \text{... (5)}$$

The total ILCR results were used to determine potential health hazards to Thailand and Myanmar residents exposed to rice grain PAH contamination. Designated ILCR over 10^{-4} is considered a serious concern for potential health problems, such as cancer; and value between 10^{-6} and 10^{-4} ILCR is considered to have a low-risk level, and an ILCR below 10^{-6} is considered safe.

RESULTS AND DISCUSSION

Concentration and Profile of PAHs from Rice Grain

In this study, 31 rice grain samples were collected from the agricultural areas of Thailand and Myanmar. The total concentrations of 12 PAHs ($\sum_{12}$PAHs: sum of Phe, An, Fluo, Pyr, B[a]A, Chry, B[b]F, B[k]F, B[a]P, Ind, D[a,h]A, and B[g,h,i]P) in these samples were qualitatively and quantitatively assessed. The total PAH concentrations of Thailand (*n* = 11) ranged from 0.09 to 37.15 $\mathrm{ng.g^{-1}}$, with an average value of 18.22 ± 11.76 $\mathrm{ng.g^{-1}}$. In this study, the $\sum_{12}$PAHs had the highest value at TH2, followed by TH5, TH10, and TH, with values of 37.15, 37.00, 25.81, and 22.30 $\mathrm{ng.g^{-1}}$, respectively (Table 2, Fig. 2a). The TH2 station is located in the Lamphun Province, northern Thailand. Very few studies have examined PAH content in Lamphun Province in recent years. However, the area of Lamphun Province is adjacent to Chiang Mai Province, where considerable research has been conducted on PAHs. The sample study of ChooChuay et al. (2020) reported that the major sources of $\mathrm{PM_{2.5}}$-bound PAHs could be attributed to the incomplete combustion of biomass, petroleum fuels, and coal, particularly vehicular exhaust. Rice grains from Myanmar (*n* = 20) had a total PAH concentration between 0.07 to 150.73 $\mathrm{ng.g^{-1}}$, with an average of 34.70 ± 40.57 $\mathrm{ng.g^{-1}}$. The $\sum_{12}$PAHs exhibited the decreasing trend of MM3, MM1, MM2, and MM20, with values of 150.73, 106.74, 80.54, and 61.60 $\mathrm{ng.g^{-1}}$, respectively. The first three highest concentrations were detected in a similar area in the

Nyaung-U District in the Mandalay Division of Myanmar. Overall, the results showed that Thailand and Myanmar had similar PAH concentrations (Table 2, Fig. 2a, and Fig. 2c). However, when comparing these values with the total concentrations of PAH detected in other plants, the values were generally lower than those found in lettuce grown near the smelter (300-920 ng.g^{-1}; Larsson & Sahlberg 1982), kale grown near the highway (500 ng.g^{-1}; Brorstrom-Lunden & Skarby 1984), sugar maple grown in urban areas of the USA (500–1,100 ng.g^{-1}; Simonich & Hites 1995), and grass grown in urban areas in the UK (153 ± 8 ng g^{-1}; Meharg et al. 1998); however, the values are similar to that of rice grown in Nanjing, Jiangsu Province, China (80.56-195.97 ng.g^{-1}, Lu et al. 2021).

Four groups of PAH molecular structures were identified in this investigation. Three-ring PAHs (i.e., Phe and An) are acutely toxic with low molecular weights (LMW-PAHs). High-molecular-weight PAHs (HMW-PAHs) comprise four-ring PAHs (i.e., Fluo, Pyr, B[a]A, and Chry), five-ring PAHs (i.e., B[b]F, B[k]F, B[a]P, and D[a,h]A), and six-ring PAHs (i.e., Ind and B[g,h, i]P), which are generally considered as genetic toxins (Abdel-Shafy & Mansour 2016, Lu et al. 2021). Additionally, HMW-PAHs are partially stable and difficult to degrade (Duan et al. 2015, Lu et al. 2021). Fig. 2b shows that the five-ring PAHs were the most abundant group in the rice grain samples of Thailand, ranging from 32% to 97% with an average of 78%, followed by four-ring (12%), three-ring (9.5%), and six-ring (0.5%) PAHs. Meanwhile, PAHs detected in the rice grains of Myanmar showed the highest content of the five-ring group, with abundance ranging from 4% to 98%, with an average of 64.02%. The second most abundant PAH group was the six-ring group (15.22%), followed by four-ring (13.58%) and three-ring groups (7.18%) (Fig. 2d). Miguel et al. (1998) suggested that HMW-PAHs are derived from gasoline and light-duty gasoline vehicles, including gasoline and oil burning at high temperatures. These previous studies are consistent with the results of Marr et al. (1999), who found that four-and five-ring PAHs dominate light-duty vehicles. In addition, incineration can release reasonably high levels of pyrene and fluoranthene (Smith & Harrison 1998).

The TEQs of PAHs in rice grains collected from Thailand and Myanmar were computed using the TEQs of 12 PAHs as TEFs (Table 2). TEQs (12 PAHs) in Thailand samples varied from 0.01 ± 0.00 to 3.60 ± 1.00 ng.g^{-1} with an average of 1.67 ± 1.20 ng.g^{-1}, whereas that in Myanmar samples ranged from N.D. to 121.89 ± 33.70 ng.g^{-1} with an average of 16.75 ± 36.83 ng.g^{-1}. Furthermore, the TEQs of 7 PAHs (B[a]A, Chry, B[b]F, B[k]F, B[a]P, Ind, and D[a,h]A) which are important carcinogens, were discovered. The TEQs of 7 PAHs were similar to those of 12 PAHs; however, the results in Thailand revealed that B[k]F had the greatest TEQs, followed by D[a,h]A and B[a]P with values of 1.51 ± 1.19, 0.07 ± 0.07, and 0.05 ± 0.08, respectively. For Myanmar samples, the highest TEQs were detected for D[a,h]A, followed by B[b]F and B[a]P with values 295.85 ± 36.33, 23.63 ± 1.29, and 8.672 ± 0.58, respectively. Very few studies have been conducted on rice-grain-based TEQ. The Canadian soil quality recommendations created by the Council of Canadian Environment Ministers (CCME) for the protection of the environment and human health are most likely the closest standard setting, which specifies an acceptable TEQ threshold of 7 PAHs of 600 ng.g^{-1} (CCME 2007, Zhong et al. 2012, Chen et al. 2018). In this investigation, the TEQ readings of rice grain samples from Thailand and Myanmar were below the recommended levels.

Source Apportionment via Diagnostic PAH Isomer Ratios

The major sources of PAHs in rice grains sampled from Thailand and Myanmar can be classified into petrogenic and pyrolytic processes. Petrogenic processes involve PAHs formed at low temperatures, for example, the slow maturation of organic materials and the environmental effects of crude oil spills (Abdel-Shafy & Mansour 2016). The incomplete combustion of biomass and fossil fuels occurs during pyrolytic reactions. It also naturally occurs during volcanic eruptions, wildfires, and brush fires under conditions of high temperature and limited oxygen. Six diagnostic binary ratios of PAH isomer pairings were employed in this work. They were displayed in Fig. 3, showing the Flou/(Flou+Pyr) ratio versus B[a]A/(B[a]A+ Chry) ratio and An/(An+Phe) ratio versus Ind/(Ind+ B[g,h, i]P. Cross-plots showing the ratios of Flou/(Flou+Pyr) and B[a]A/(B[a]A+ Chry) can be used to determine the possible source of PAH contamination in rice grains. The results showed that most of the PAHs in the grain samples came from incomplete combustion of various sources.

Furthermore, the individual Flou/(Flou+Pyr) ratios reveal that samples collected from stations TH2, TH3, TH5, TH8, TH10, and TH11 in Thailand may be contaminated by incomplete combustion of biomass, fossil fuel, coal, and possibly diesel emissions. The samples TH1, TH7, and TH9 were formed from petrogenic processes. However, TH4 and TH6 from Thailand could not use the diagnostic binary ratios to determine the source due to the absence of some PAHs in the grain samples of these stations. The results in Myanmar showed that the PAHs of almost all the rice grain samples originated from incomplete combustion, similar to those from Thailand, except for the MM1 station, which originated from

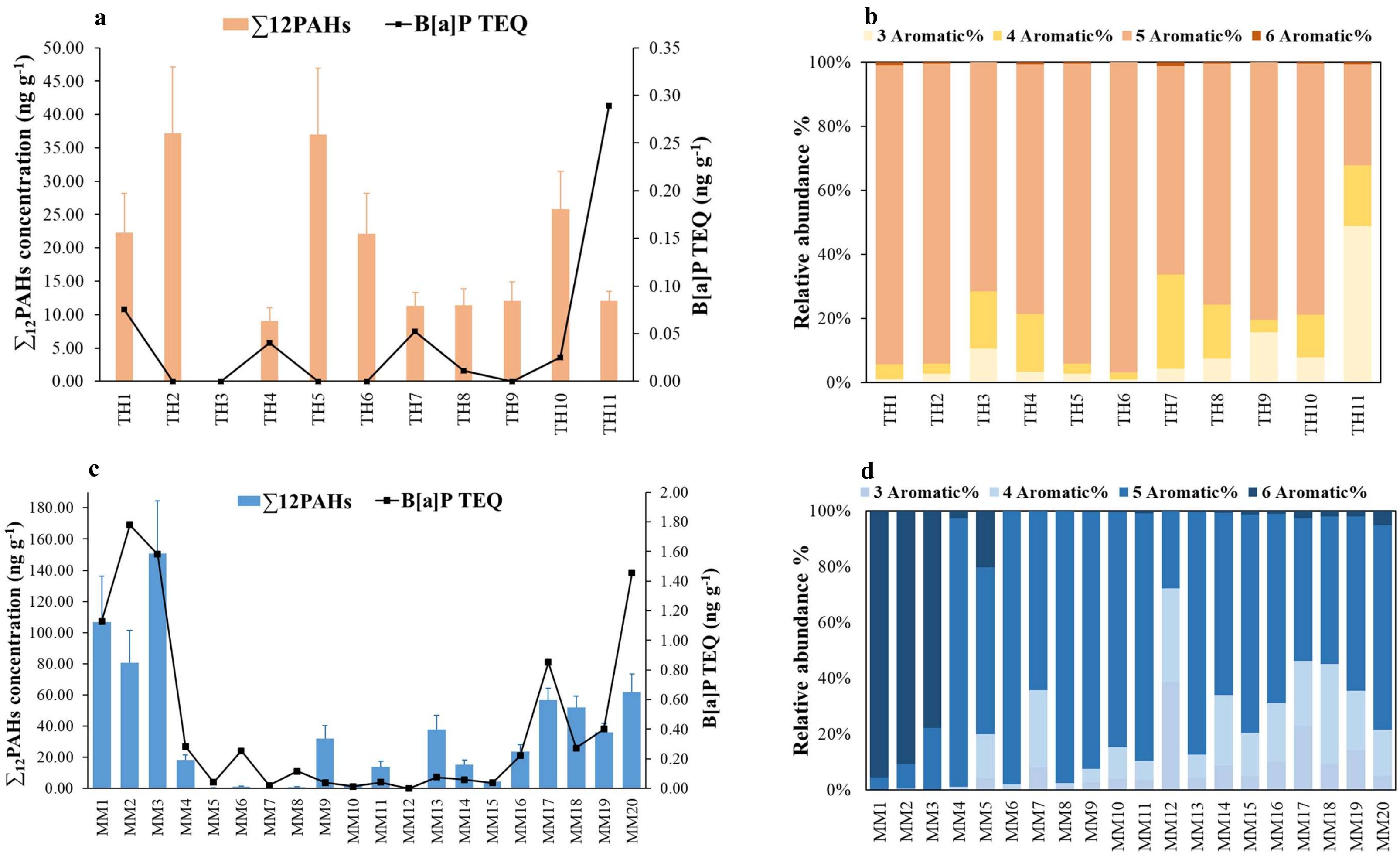

Fig. 2: (a) Total PAH concentrations of 12 PAHs ($\sum_{12}$PAH) and B[a]P toxic equivalence quotient (TEQ) in the rice grain samples of Thailand. (b) Distribution patterns of % contribution and classification of 3–6 ring PAHs in the rice grain samples of Thailand. (c) Total PAH concentrations of 12 PAHs ($\sum_{12}$PAH) and B[a]P TEQ in the rice grain samples of Myanmar and (d) distribution patterns of % contribution and classification of 3–6 ring PAHs in the rice grain samples of Myanmar.

Table 2: The total 12 PAH concentrations and toxic equivalent concentrations.

Sampling sites	$\sum_{12}$PAHs [ng.g^{-1}]	$\sum_{12}$TEQ [ng.g^{-1}]	$\sum_{7}$TEQ [ng.g^{-1}]	Sampling sites	$\sum_{12}$PAHs [ng.g^{-1}]	$\sum_{12}$TEQ [ng.g^{-1}]	$\sum_{7}$TEQ [ng.g^{-1}]
MM1	106.74 ± 29.32	103.43 ± 29.40	103.43 ± 38.45	TH1	22.30 ± 5.89	2.41 ± 0.59	2.41 ± 0.76
MM2	80.54 ± 20.94	75.36 ± 21.03	75.36 ± 27.47	TH2	37.15 ± 9.99	3.60 ± 1.00	3.60 ± 1.31
MM3	150.73 ± 33.68	121.89 ± 33.70	121.89 ± 43.98	TH3	0.09 ± 0.02	0.01 ± 0.00	0.01 ± 0.00
MM4	18.32 ± 3.28	2.22 ± 0.33	2.22 ± 0.38	TH4	9.04 ± 1.96	0.77 ± 0.20	0.77 ± 0.26
MM5	0.36 ± 0.03	0.08 ± 0.01	0.08 ± 0.02	TH5	37.00 ± 9.95	3.58 ± 1.00	3.58 ± 1.30
MM6	1.25 ± 0.24	0.35 ± 0.07	0.35 ± 0.09	TH6	22.13 ± 6.04	2.16 ± 0.61	2.16 ± 0.79
MM7	0.15 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	TH7	11.31 ± 2.00	0.92 ± 0.20	0.92 ± 0.26
MM8	0.80 ± 0.16	0.18 ± 0.04	0.18 ± 0.04	TH8	11.44 ± 2.42	0.95 ± 0.25	0.9 5± 0.32
MM9	31.82 ± 8.37	3.03 ± 0.84	3.03 ± 1.10	TH9	12.09 ± 2.78	0.98 ± 0.28	0.97 ± 0.37
MM10	2.42 ± 0.57	0.22 ± 0.06	0.22 ± 0.08	TH10	25.81 ± 5.66	2.21 ± 0.57	2.20 ± 0.74
MM11	13.93 ± 3.50	1.35 ± 0.35	1.35 ± 0.46	TH11	12.02 ± 1.50	0.75 ± 0.12	0.73 ± 0.15
MM12	0.07 ± 0.01	N.D	N.D				
MM13	37.66 ± 9.30	3.45 ± 0.94	3.45 ± 1.22				
MM14	15.36 ± 2.78	1.17 ± 0.28	1.16 ± 0.37				
MM15	4.32 ± 0.94	0.42 ± 0.09	0.42 ± 0.12				
MM16	23.62 ± 4.33	2.08 ± 0.44	2.06 ± 0.56				
MM17	56.76 ± 7.61	4.76 ± 0.80	4.70 ± 0.97				
MM18	51.81 ± 7.32	4.00 ± 0.75	3.98 ± 0.94				
MM19	35.81 ± 5.92	3.14 ± 0.61	3.11 ± 0.77				
MM20	61.60 ± 11.62	7.75 ± 1.26	7.72 ± 1.52				
Myanmar (n = 20)	34.70 ± 40.57	16.75 ± 36.83	16.74 ± 36.83	Thailand (n = 11)	18.22 ± 11.76	1.67 ± 1.20	1.66 ± 1.20

a petrogenic source. The same is true for the B[a]A/(B[a]A+ Chry) ratio, which separates the origin of PAHs into two major contributors: vehicular combustion and mixed sources. The values > 0.35 indicate combustion sources, which consisted of stations TH1, TH4, TH8, TH9, TH10, and TH11 (samples collected from Thailand) and MM1, MM2, MM4, MM5, MM7, MM15, MM16, MM17, MM18, MM19, and MM20 (samples collected from Myanmar). TH2, TH5, TH7, MM3, MM9, MM10, MM11, MM13, and MM14 stations had values ranging from 0.2 to 0.35, indicating the possibility of a mixed origin from incomplete combustion of multiple sources or petroleum. The ratios of An/(An+Phe) versus Ind/(Ind+ B[g,h, i]P) revealed the origins of the petrogenic and pyrogenic PAHs in the rice grain samples (Fig. 3b). The Ind/(Ind+ B[g,h, i]P) ratio, in particular, showed that the majority of the PAHs were produced during the burning of petroleum. Additionally, the values of B[a]P/B[g,h, i]P > 0.6 indicate that traffic emissions form PAHs. The results confirmed that the source of approximately 81% of the stations capable of measuring PAHs was traffic-originated.

Source Identification Based On Principal Component Analysis (PCA)

In this study, we employed PCA to determine the sources of 12 PAHs in 31 samples from Thailand and Myanmar. PCA is the most extensively used statistical technique in research. PCA makes complex systems easier to understand. The major goal of this study was to save maximum original data as feasible while reducing the number of variables. The varimax rotation technique and Kaiser standardization were employed in PCA to determine the origins of PAHs in Thailand (Table 3). Principal components of four eigenvector elements explain the scaled data, accounting for 85.98% of the total variance. 32.88% of the variance was explained by the first principal component (PC1). This factor was primarily loaded on the four-ring elements Pyr, B[a]A, and Chry, with the exception of B[g,h, i]P. Many sources are associated with pyrolysis and incomplete combustion of fuels, particularly gasoline-powered cars (Duval & Friedlander 1981, Khalili et al. 1995, Ravindra et al. 2008, Deelaman et al.2020). The PCA result was associated with the ratio of B[a]P/B[g,h, i]P, indicating > 80% of the contaminated samples were caused by traffic emissions. The second principal component (PC2), which was primarily loaded on Phe, An, and B[a]P, the three- and five-ring PAHs produced from various sources linked to emissions from heavy-duty vehicles, accounted for 27.51% of the total variation. This is consistent with the studies of Caricchia et al. (1999), Fang et al. (2004), and Ravindra et al.

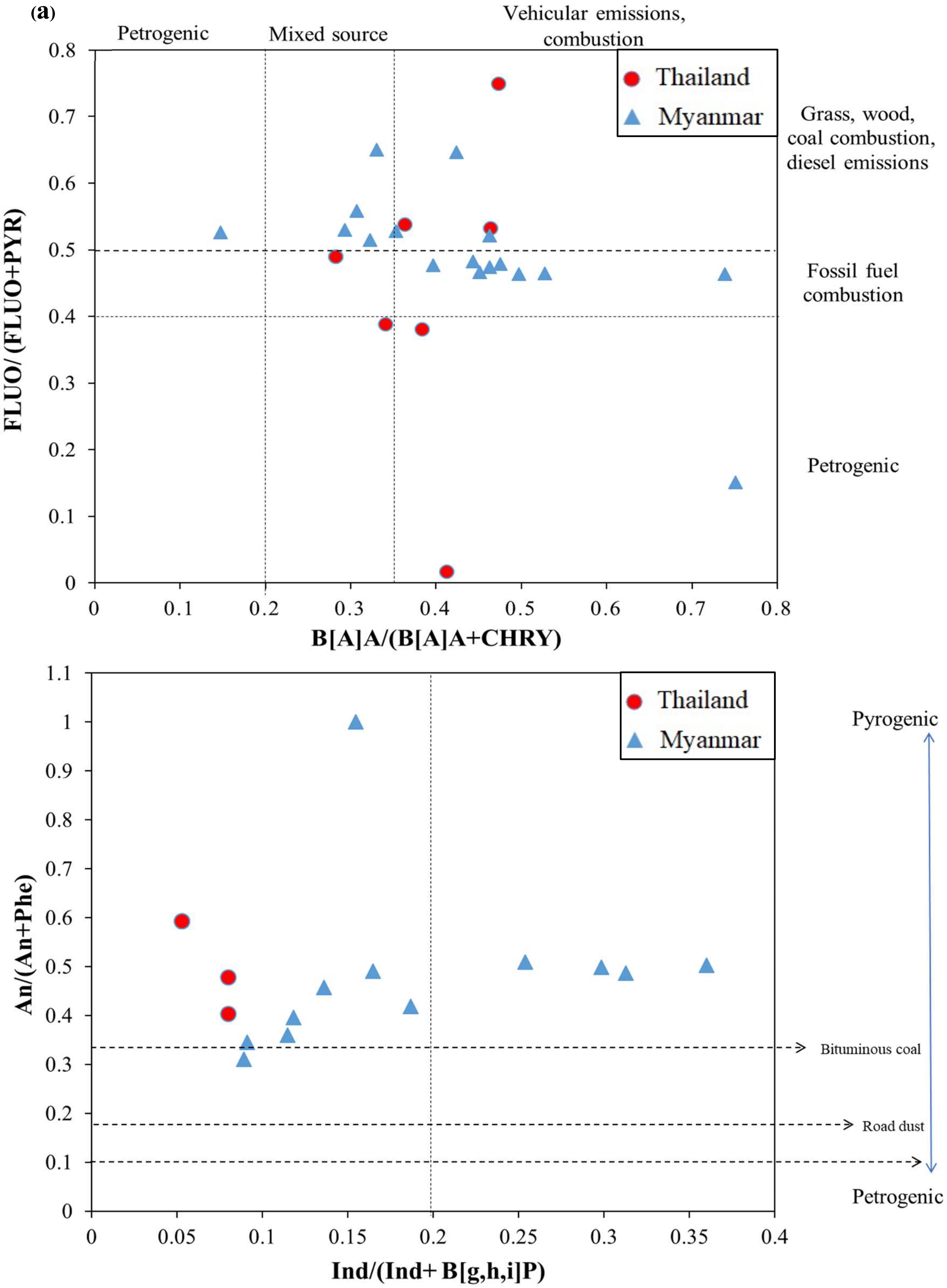

Fig. 3: Cross-plot for the ratios: (a) Flou/(Flou+Pyr) versus B[a]A/(B[a]A+ Chry) and (b)An/(An+Phe) versus Ind/(Ind+ B[g,h, i]P)in the sample from Thailand and Myanmar.

(2008), who reported that phenanthrene and anthracene are high load factors owing to diesel emissions. However, B [a] P originates from fossil fuel-based power plants (Ravindra et al. 2008). The significant loading of B[k]F and D[a,h]A, which are five-ring PAHs, explained 13.37% of the total variance in the third main component (PC3). Therefore, PC3 is associated with incomplete biomass combustion and is considered a pyrogenic source. Freeman and Cattel (1990) reported that the indicator of biomass combustion (e.g., wood and grass) is D[a,h]A. Burning biomass is a common technique in tropical Asia for clearing land and converting it for other uses (Hyer & Chew 2010, Tsay et al.2016, Punsompong et al.2021). In Thailand, a country with a large agricultural sector in Southeast Asia, most farmers burn agricultural waste to make way for new crop cycles (Pani et al. 2018, Vadrevu et al. 2019, Punsompong et al. 2021). However, Thailand has different land-use patterns, and agricultural burning behavior depends on the region. In the northern and central regions, most biomass is combusted with rice straw and sugar cane leaves. In the south, there is very little burning of agricultural waste (Punsompong et al. 2021). Therefore, PC3 can be considered to indicate biomass combustion. Finally, the fourth principal component (PC4) was strongly connected to B[b]F and accounted for only 12.23% of the total variance. A high factor loading of B[b]F has been suggested for stationary emission sources (Kulkarni & Venkataraman 2000, Ravindra et al.2006). B[b]F has also been associated with moderate oil burns (Harrison et al. 1996, Ravindra et al. 2008). Thus, it could be interpreted that PC4 represents an industrial plant or incinerator. This result matches the current scenario. Thailand is a middle-income country developing into a high-income country using new technology and innovation-based economic engines (Ministry of Industry 2021). Many industries have benefited from government support resulting in increased consumption of fossil fuels.

Two eigenvector-principal components could account for the source of PAHs found by PCA in Myanmar, controlling 76.38% of the total variance (Table 4). The first principal component (PC1) describes 60.58% of the total variance, which is due to the strong loading of three- to six-ring PAHs, such as Phe, An, Fluo, Pyr, B[a]A, Chry, B[b]F, Ind, and B[g, h, i]P. As the low- to moderate-molecular-weight PAHs (3-4 rings) originated from the use of diesel fuel in vehicles, such as trucks, trains, and ships, we interpret that PC1 may represent incomplete combustion from energy-dense secondary fuel for light-duty vehicles (gasoline) and heavy-duty vehicles (diesel fuel). Diesel emissions have also been reported to have a high load factor for Fluo, Phe, An, and Pyr (Caricchia et al. 1999, Ho et al. 2002, Fang et al.2004, Ravindra et al.2006). In addition, the combustion of gasoline is reportedly associated with high-molecular-weight compounds (5–6 rings), such as B[b]F and Ind (Harrison et al.1996, Ravindra et al. 2006, Deelaman et al. 2020b). Therefore, PC1 may suggest that incomplete fuel combustion is the source of PAH formation. In comparison, 15.80% of the total variation was explained by the second principal component (PC2). Only two compounds, B[a]P and D[a,h]A, two high-molecular-weight PAHs with five rings, comprised most of the factor's weight. The indicator compound in PC2 was D[a,h]A, similar to PC3 in Thailand. PC2 was likely a reasonable representative of biomass burning in Myanmar

Table 3: Principal component analysis results for PAHs in the rice grains sampled from Thailand.

PAH composition	Principle component (PC)			
	1	2	3	4
Phe	-0.050	**0.967**	0.036	-0.031
An	-0.056	**0.974**	-0.114	-0.025
Fluo	0.375	0.457	0.327	0.345
Pyr	**0.923**	0.262	0.049	-0.106
B[a]A	**0.896**	0.059	-0.022	0.332
Chry	**0.968**	0.079	0.061	0.076
B[b]F	0.022	-0.105	0.071	**0.836**
B[k]F	-0.158	-0.294	**0.859**	-0.081
B[a]P	0.148	**0.914**	-0.053	0.032
Ind	0.669	-0.293	0.176	-0.567
D[a,h]A	0.149	0.145	**0.824**	0.129
B[g,h,i]P	**0.830**	-0.304	-0.147	-0.418
Total variance [%]	32.883	27.507	13.367	12.225
Cumulative [%]	32.883	60.390	73.757	85.982
Estimated source	Gasoline-powered vehicles	Heavy-duty vehicles	Biomass burning	Stationary point sources

Extraction method: Principal component analysis.
Rotation method: Varimax with Kaiser normalization.

Table 4: Principal component analysis results for PAHs in the rice grains sampled from Myanmar.

PAH composition	Principle component (PC)	
	1	2
Phe	**0.866**	-0.086
An	**0.833**	-0.037
Fluo	**0.958**	-0.136
Pyr	**0.962**	-0.112
B[a]A	**0.951**	-0.058
Chry	**0.942**	-0.102
B[b]F	**0.800**	0.301
B[k]F	-0.183	-0.014
B[a]P	0.247	**0.946**
Ind	**0.839**	0.201
D[a,h]A	-0.242	**0.884**
B[g,h,i]P	**0.834**	0.187
Total variance (%)	60.576	15.801
Cumulative (%)	60.576	76.377
Estimated source	Incomplete combustion	Biomass burning

Extraction Method: Principal component analysis.
Rotation Method: Varimax with Kaiser normalization.

(Nepstad et al. 1999, Lambin et al. 2003). In Myanmar, forests reportedly account for approximately 48% of the total land area, and most have an agriculture-based economy. In both urban and rural settings, wood is a significant energy source for households and some industries (Khaing 2012, Tun et al. 2019). Over half of Myanmar's entire energy supply in 2012–2013 came from biomass, comparable to 10 million tons of oil (Tun & Juchelková 2019). There have also been reports of biomass burning being associated with the threat of forest clearing for agriculture and infrastructure development. Based on the above reasons, we conclude that PC2 in Myanmar may originate from biomass combustion.

Identifying the sources of PAHs in rice grains revealed that incomplete combustion of petroleum from various activities was the key source of PAHs in Thailand and Myanmar, followed by biomass combustion. The results would aid future agricultural food product control and environmental management planning.

Health Risk Assessment of PAHs in Rice Grains

According to the health risk assessment of PAHs in rice grain samples from Thailand, the mean values for inhalation of $\text{ILCR}_{\text{inhalation}}$ for children and adults in all grain samples were 33.61×10^{-14} and 4.02×10^{-13}, respectively. The ILCR results were less than the baseline value, indicating that PAHs obtained from rice grain exposure by inhalation are at safe levels. The mean values of $\text{ILCR}_{\text{Dermal}}$ were 1.08×10^{-8} and 9.21×10^{-9}, respectively, and $\text{ILCR}_{\text{Ingestion}}$ values were

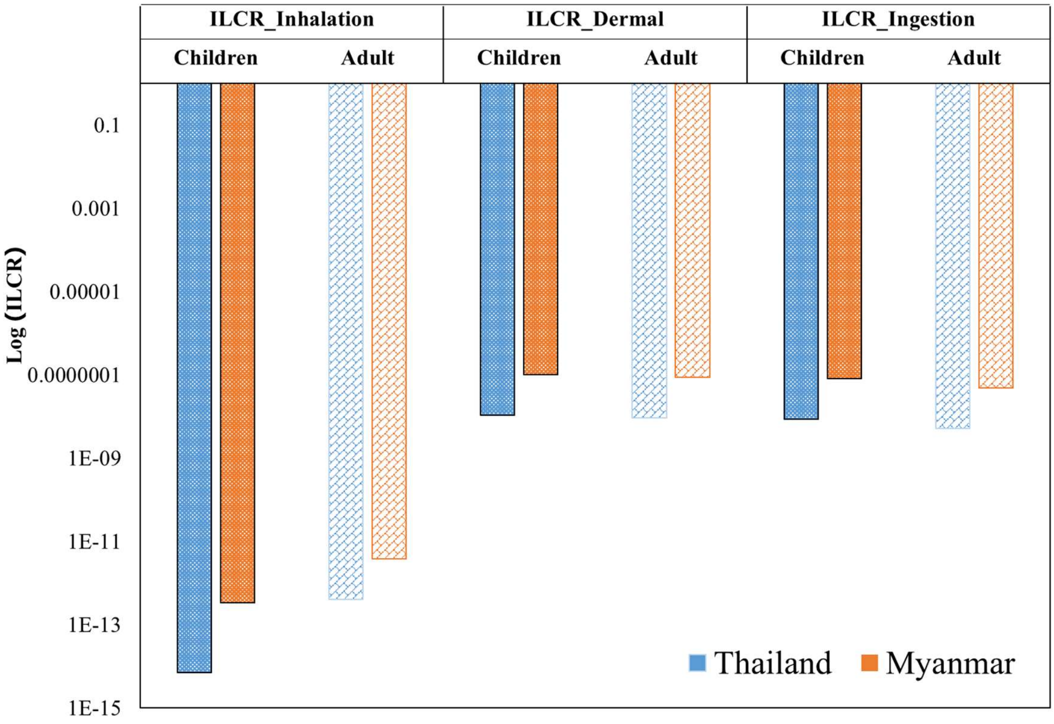

Fig. 4: Logarithmic comparisons of different exposure pathways to the ILCRs between Thailand and Myanmar.

8.68×10^{-9} and 5.18×10^{-9} for children and adults, respectively. However, these results showed that PAH exposure pathways for rice grain samples collected from the Thailand area through dermal contact were at the highest risk, followed by ingestion and inhalation, with the ILCR value higher in children than in adults (Fig. 4). All these results showed that ILCRs values in Thailand were 1.95×10^{-8} for children and 1.44×10^{-8} for adults, which were lower than the standards set and are considered to be safe levels.

All PAHs contributed to the health risk of rice grains in Myanmar, with mean $ILCR_{inhalation}$ values of 3.39×10^{-13} and 3.78×10^{-12} for children and adults, respectively. $ILCR_{Dermal}$ mean values for children and adults were 1.02×10^{-7} and 8.65×10^{-8} respectively, whereas $ILCR_{Ingestion}$ mean values were 8.16×10^{-8} and 4.87×10^{-8}, respectively. In addition, the $ILCR_{inhalation}$ was higher in adults than children; however, the ILCR of ingestion and exposure was higher in children than in adults. The total health risks (ILCRs) from three main pathways (inhalation, dermal contact, and ingestion) showed a mean value of 1.83×10^{-7} and 1.35×10^{-7} for children and adults, respectively. According to these findings, the cumulative lifetime cancer risk from rice grains obtained from Myanmar was lower than the baseline set for safe values.

CONCLUSIONS

This study used multivariate descriptive statistical methods to examine the PAH composition in rice grains. We determined the origin of PAH in rice grains from Thailand and Myanmar using diagnostic ratios and PCA together with its toxicity and health risk assessment. The findings can be summarized as follows: (i) The level of PAHs ($\sum$12PAHs) in Thailand varied from 0.09 to 37.15 $ng.g^{-1}$ with an arithmetic mean of 18.22 ± 11.76 $ng.g^{-1}$, whereas that in Myanmar varied from 0.07 to 150.73 $ng.g^{-1}$ with an arithmetic mean of 34.70 ± 40.57 $ng.g^{-1}$, which are relatively low compared to previous studies. (ii) The attribution of PAHs in Thailand and Myanmar rice grains was based on diagnostic ratios and PCA. The results of the diagnostic ratio show that pyrogenic sources were the main sources of PAHs in the rice grain samples from Thailand and Myanmar. In addition, the results indicated that the majority of PAHs in the samples at all sampling sites were formed by incomplete combustion of petroleum products and biomass combustion, consistent with the PCA results of PAHs in both Thailand and Myanmar, indicating that they primarily originated from pyrogenic sources (gasoline-powered vehicles, heavy-duty vehicles, and biomass). (iii) The rice grains of Thailand and Myanmar contaminated with PAH congeners exhibited relatively low toxicity. However, the increased lifetime cancer risk assessment results showed that both adults and children had low cancer risks that fell under the USEPA allowed range. Although PAHs in rice have a low impact on crops and human health in Thailand and Myanmar, as rice is predominantly consumed in these areas, preventive practices are required to check the PAH content.

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