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Pyrethroid and Heavy Metal Residues in Different Coffee Bean Preparation Processes and Human Health Risk Assessments via Consumption

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Abstract

This study was conducted to determine the concentrations of pyrethroid and metal residues in 50 coffee bean samples of various origins. The samples included green coffee beans, roasted beans, brewed coffee drinks, and coffee sludge. Three processes were used to prepare the samples: drying, semi-washing, and washing. Three synthetic pyrethroid insecticides (flumethrin, cypermethrin, and cyfluthrin) and nine heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) were subsequently analyzed using the modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for insecticides and acid digestion method for heavy metals. The pyrethroids and heavy metals were quantified using a gas chromatograph equipped with a micro-electron capture detector and a flame atomic absorption spectrophotometer, respectively. According to the results, both pyrethroids and heavy metals, except for Cr, were predominantly found in green coffee beans. The pyrethroid and heavy metal with the highest concentrations were cyfluthrin 2 (0.84 ± 0.25 µg/g) and Cu (42.56 ± 18.12 µg/g), respectively. Pyrethroid insecticides were not detected in brewed coffee drinks, and the heavy metals were below the acceptable daily intake level. Risk estimations for daily coffee intake using the health risk indices and target hazard quotients of average and the 97.5th percentile of Thai coffee drinkers were less than 1. This indicated that the coffee drinks in the studied samples did not pose potential health risks.

Keywords: Coffee; Health risk; Heavy metals; Pyrethroid; Residues

1. Introduction

Coffee is a popular beverage worldwide. It contains several beneficial antioxidants and is one of the richest chlorogenic acid sources (Clifford, 1999). However, coffee drinking can potentially expose humans to multiple toxicants. These toxicants include pesticides, heavy metals, organic solvents, pharmaceutical agents, and byproduct contaminants from thermal processes, such as acrylamide (Studer *et al.,* 2004). These hazardous chemicals are commonly used to protect coffee trees from pests and diseases during cultivation and manufacturing processes (Hillocks *et al.,* 1999). Although coffee beans are roasted, pesticide residues are still present. Recent studies have revealed that coffee beans and coffee wastes are contaminated by numerous pesticides and metals (Dias *et al.,* 2013; Mekonen *et al.,* 2015; Yang *et al.,* 2011).

Pesticides and insecticides are used on coffee crops by directly applying them to the soil or to the aerial parts of the plants. The chemical classes generally used include organophosphates, pyrethroids, carbamates, chlorinated cyclodienes, and organotins (Reis *et al.,* 2015). Among these, the most preferred class is pyrethroids, which accounts for one-fifth of the total insecticide global market (Zhang, 2018), because of their low toxicity to mammals but high toxicity to insects (Ravula and Yenugu, 2021). Pyrethroids are synthetic analogs of pyrethrin found in *Tanacetum cinerariifolium* (Asteraceae) or pyrethrum daisy. The commonly used pyrethroids in coffee culture are cypermethrin, esfenvalerate, fenpropathrin, permethrin, and cyfluthrin (de Queiroz *et al.,* 2018). Humans can be exposed to these pyrethroids via inhalation, dermal contact, and ingestion (the primary route) (Saillenfait *et al.,* 2015). Exposure can deteriorate human health by affecting the reproductive, urinary, immune, and respiratory systems. Although coffee consumption may result in harmful health effects, data regarding this are still limited.

Heavy metals are another group of toxicants found in coffee beans. They naturally occur or result from anthropogenic activities (Giangrosso *et al.,* 2016; Vareda *et al.,* 2019). Heavy metals, either trace or toxic, are widely dispersed in the environment and enter the food chains at different concentrations. Coffee plants can absorb these metals through their roots and translocate them to shoots and grains (Qais *et al.,* 2014). Lifetime consumption of contaminated beans may have adverse effects on human health.

The objectives of this study were 1) to determine the residues of pyrethroid insecticides (flumethrin, cypermethrin, and cyfluthrin) and heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) in various forms of coffee such as green, roasted, brew, and sludge, originating from green coffee beans prepared via three processes (dry, semi-washed, and washed) and 2) to evaluate the human health risks of insecticide-contaminated and metal-contaminated coffee drinks based on the estimated daily intake (EDI), health risk index (HRI), and target hazard quotient (THQ). The results of this study will provide useful baseline data on the levels of contaminants in coffee and human health risks associated with coffee consumption.

2. Materials and Methods

2.1 Sample collection and preparation

Fifty green coffee bean samples (1 kg each) were randomly purchased from local markets in Bangkok, Thailand. These samples originated from Asia (China, India, Loas, Thailand, and Vietnam), America (Columbia, Costa Rica, Honduras, and Mexico), and Africa (Ethiopia and Uganda). All samples were divided into three groups according to their preparation processes: drying, semi-washing, and washing. Each sample was aliquoted into two equal subsamples. One subsample was ground and homogenized with a miller and served as the green coffee bean sample. Another subsample was roasted at 230 °C until the coffee aroma was generated (Mekonen *et al.,* 2015). The roasted beans were ground into a fine powder with a stainless-steel blender, and 10 g of this sample was collected and added to 180 mL of hot water (96 °C). The brewed coffee drink was then collected using the paper-drip technique. The coffee sludge remaining in the filtered paper was also collected and oven dried at 65 °C before storage. Green coffee beans from organic cultivation in Thailand were used as the blank and spiked samples. They were prepared using processes similar to those used for preparing the samples. All samples were stored at −20 °C until chemical analysis.

2.2 Sample extraction and clean-up

All chemicals and reagents for extraction, clean-up, and analysis had ≥ 99% purity and were analytical or pesticide grade. Standard pyrethroid insecticides and individual heavy metal standard solutions were purchased from Supelco® Sigma-Aldrich (St. Louis, MO, USA).

2.2.1 Pyrethroid residues

To examine the differences in the amount of pyrethroid insecticide residues among coffee bean preparation processes, the extraction and clean-up of green coffee beans and processed coffee samples (roasted coffee powder, brewed coffee drink, and coffee sludge) were performed using a modified QuEChERS method combined with a dispersive solid-phase extraction (d-SPE) clean-up method (Özdemir *et al.,* 2019). Different salts (NaCl, $C_2H_3NaO_2$, $C_6H_9Na_3O_9$, and $C_2H_3NaO_2$) and their quantities were investigated to optimize the extraction method. The salts and their quantities with the best recovery rates were selected for the pyrethroid analysis. The extraction and clean-up of green coffee beans and processed coffee samples were performed as follows:

Green coffee beans and brewed coffee drink: Green coffee beans (2.5 g) or brewed coffee drinks (10 mL) were added to 10 mL acetonitrile and shaken by hand for 1 min. The samples were then mixed with 4 g of $MgSO_4$ and 1.5 g of $C_2H_3NaO_2$ and shaken for 1 min. They were subsequently centrifuged at 5,000 rpm for 5 min. The upper organic layer (2 mL) was taken and translocated to a d-SPE tube containing 600 mg of $MgSO_4$, 300 mg of PSA, and 100 mg of alumina. The extract was shaken for 1 min and then centrifuged. After centrifugation, 200 µL of the upper layer was collected for the analysis of pyrethroid residues using gas chromatography equipped with a micro-electron capture detector (GC-µECD).

Roasted coffee powder and coffee sludge: Fine roasted coffee powder (2.5 g) of coffee sludge (2.5 g) were added to 10 mL of acetonitrile and shaken for 30 s. Then, 4 g of $MgSO_4$, 1 g of NaCl, 1 g of $C_6H_9Na_3O_9$, and 0.5 g of $C_6H_5Na_3O_7$ were added and shaken for 30 s. The samples were centrifuged at 5,000 rpm for 5 min. The upper organic layer (2 mL) was taken and placed into a d-SPE tube containing 600 mg of $MgSO_4$, 100 mg of PSA, and 100 mg of alumina; shaken for 1 min; and centrifuged at 5,000 rpm for 5 min. After centrifugation, 200 µL of the upper layer of each sample was taken for GC-µECD analysis.

2.2.2 Heavy metals

The acid digestion method was used to extract nine metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) from all samples. The concentration of each metal was determined using FAAS. The digestion procedures of each sample type were as follows:

Green coffee beans, roasted coffee powder, and coffee sludge: Approximately 0.5 g of individual samples were digested with 5 mL mixture of 70% HNO_3:70% $HClO_4$ (3:1 v/v) at 180 °C for at least 3 h. The digested solution was maintained under this condition until a clear solution indicating complete digestion was observed. Each sample was filtered using No. 4 Whatman filter paper (Dassel, Germany), adjusted to 25 mL using ultra-high purity water, and stored at 4 °C until FAAS analysis.

Brewed coffee drink: Briefly, 25 mL of each sample was digested with 5 mL of 70% HNO_3 at 180 °C for at least 3 h. The digested samples were cooled to room temperature (25 °C) and then filtered through a No. 4 Whatman filter paper. The solution volume was adjusted to 25 mL using ultra-high-purity water and stored at 4 °C until FAAS analysis.

2.3 Sample analysis

2.3.1 Gas chromatographic analysis

Pyrethroid residue analyses were performed using GC-µECD (GC 7890 B, Agilent Technologies, Germany). The system was equipped with a 30 m × 0.32 mm × 0.25 µm fused silica capillary column (HP-5, Agilent Technologies, Germany). High-purity (99.999%) helium and nitrogen were used as carrier gas and make up gas at a constant flow rate of 20 mL/min and 60 mL/min, respectively. The GC oven was operated as follows: an initial temperature of 150 °C was maintained for 1 min, and the temperature was then increased to 255 °C at a rate of 15 °C/min and further increased to 300 °C at a rate of 20 °C/min and held for 5 min. The injector and detector temperatures were set at 260 °C and 315 °C, respectively. The total run time was 28 min per sample. One microliter of each sample was injected into the GC system in the splitless mode. Pyrethroid concentrations were determined using relevant standard calibration curves at six concentration points. Each sample was analyzed in triplicate to ensure reliable results.

The linearity, percentage recovery, precision, limit of detection (LOD), and limit of quantification (LOQ) for pyrethroid insecticides were determined using the European Commission (SANCO/12571/2013) criteria. The linearity (r^2 > 0.998) was validated for a concentration range of 0.001–1.000 µg/g using seven replicates at each concentration. The percentage recoveries were determined using spiked known pyrethroid insecticide concentrations (0.05, 0.25, and 0.50 µg/g) in each sample matrix with 10 replicates for each concentration. They were calculated by comparing the ratio of each spiked concentration to the analyzed concentration, and the percentage recoveries of the studied pyrethroids ranged from 79.29 to 102.65. Precisions (%RSD) were all below 20% (0.75% -14.90%). The LODs and LOQs ranged from 0.001 to 0.020 µg/g and 0.010 – 0.065 µg/g, respectively.

2.3.2 Heavy metal analysis

Nine heavy metal concentrations were determined using an air-acetylene flame on an FAAS system (SpectrA 240 B Agilent Technologies, Australia) equipped with a deuterium background corrector. The individual metal concentrations were calculated using the corresponding calibration curve. Each sample was analyzed in triplicates to ensure the reliable results.

The linearity (r^2 > 0.999) of calibration curves was evaluated using seven replicates of six metal concentrations of each metal. The percentage recovery of each heavy metal was determined based on their spiked known metal concentrations and all studied metals were greater than 97.5. Precisions (%RSD) were all below 20% (0.07% - 4.68%). The LODs and LOQs ranged from 0.0005 – 0.0020 µg/g and 0.001 – 0.005 µg/g, respectively.

2.4 Determination of processing factor

The processing factor (PF) for each transformation step (roasting, brewing, and sludge) was calculated as the ratio of the level of pyrethroid insecticide or metal in processed samples (roasted coffee powder, brewed coffee drink, and coffee sludge) (µg/kg) to those in non-processed samples (green coffee beans) (µg/kg) using the following equation:

$$\text{Processing factor (PF)} = \frac{\text{Level of pyrethroid insecticide or metal in processed sample (µg/kg)}}{\text{Level of pyrethroid insecticide or metal in unprocessed sample (µg/kg)}}$$

PF of <1 indicated that there was a reduction in the level of pyrethroid insecticide or metal during processing, whereas a PF of >1 indicated that there was no reduction in the level of these toxic residues (Bonnechere *et al.,* 2012). In addition, the percentage reduction (% reduction) for individual processing steps was calculated using the following equation:

$$\% \text{ reduction} = (1\text{-PF})\times 100$$

2.5 Human health risk assessment

The EDI, HRI, and THQ were used to evaluate the non-carcinogenic human health risks of consuming insecticide- and metal-contaminated coffee drinks. The carcinogenic risks due to the exposure of Cd, Co, Cr, Pb, and Ni were also calculated according to Sultana *et al.* (2017). The summation of Incremental Lifetime Cancer Risk (ILCR) of carcinogenic heavy metals were below the permissible limits ($<10^{-4}$ for multi-element carcinogens) (Tepanosyan *et al.,* 2017). The health risk was also determined among individuals with an average consumption of brewed coffee drinks and the 97.5th percentile of coffee drinkers (extreme consumers). The formulae used in calculating EDI, HRI, and THQ were as follows:

$$EDI = \frac{C \times W_F}{W_{AB}}$$

where C is the pyrethroid or metal concentration in brewed coffee samples (µg/mL), W_F is the daily average consumption of coffee drinkers only in Thailand (mL/person/day); 216.06 mL/person/day for average and 330 mL/person/day for the 97.5th percentile) (National Bureau of Agricultural Commodity and Food Standards, 2016), and W_{AB} is the average body weight (70 kg for adults).

$$HRI = \frac{EDI}{\text{ADI or RfD}}$$

where ADI is the acceptable daily intake and RfD is the oral reference dose (in the unit of mg/kg BW/day Σcypermethrin 0.010; Σcyfluthrin 0.004; Cd, 0.001; Cr, 0.003; Cu, 0.500; Fe, 0.800; Mn, 0.140; Ni, 0.020; Pb, 0.025; and Zn, 0.300) (EPA, 2019; WHO, 2018).

$$THQ = \frac{E_F \times E_D \times F_{IR} \times C}{RfD \times W_{AB} \times T_A} \times 10^{-3}$$

where E_F is the exposure frequency (365 day/year), E_D is the exposure duration (70 years), F_{IR} is the food ingestion rate (mL/person/day), RfD is the oral reference dose (mg/kg BW/day), W_{AB} is the average body weight, and T_A is the average exposure time (365 days/year × lifetime, assuming 70 years).

2.6 Data analysis

Statistical analysis was performed using R statistical software version 3.4.3 (2017-11-30). Results were shown as mean ± standard error of the mean (SEM). All data were evaluated for normality of distribution prior to one-way analysis of variance (ANOVA). ANOVA was used to test significant differences ($P < 0.05$) and similarities among insecticide residues found in coffee samples subjected to different processes. Principal component analysis (PCA) was also performed to determine the possible causes of sample contamination (Wu *et al.,* 2021; Zhao *et al.,* 2020) using factor extraction with an eigenvalue of > 1.

3. Results and Discussion

3.1 Pyrethroid residues in coffee samples

Flumethrin, cypermethrin, and cyfluthrin were predominantly found in green coffee beans that were prepared using various processes (drying, semi-washing, and washing) but not in brewed coffee drinks. The residues in green coffee beans differed significantly ($P < 0.05$) among the processing steps (Table 1). The cyfluthrin 2 (0.84 ± 0.25), cyfluthrin 3, (0.19 ± 0.04 μg/g) cypermethrin CisA (0.06 ± 0.01 μg/g), and Σcyfluthrin (1.28 ± 0.33 μg/g) concentrations in dried processed coffee beans were the highest ($p < 0.05$). In addition, the concentrations of flumethrin (0.75 ± 0.01 μg/g) and cypermethrin TransD (0.11 ± 0.05 μg/g) found in semi-washed processed coffee beans were significantly highest ($P < 0.05$). A simple washing process for easily removing these residues has been demonstrated (Ahmed *et al.,* 2011). Pesticides and insecticides are removed from the outer and silver skin of the beans after washing and semi-washing (Mekonen *et al.,* 2015). However, this residue removal may not always correlate with the water solubility of each residue. Peeling and refrigeration storage may also affect the reduction of residues (Cengiz *et al.,* 2007). The Σcypermethrin concentrations found in green coffee beans subjected to the drying process exceeded the concentration stipulated by the EU legislation (0.1 μg/g), whereas those in green coffee beans that were semi-washed and washed were greater than the concentration stipulated by Japan, USA, Ecuador, Indonesia, and Kenya regulations (0.05 μg/g) (European Commission, 2012; International Coffee Council, 2013). The Σcypermethrin concentrations in bean samples exceeded the regulatory limits in Africa and Asia. This is likely because of the widespread use of pyrethroids in these regions (Li *et al.,* 2016).

The pyrethroid residues in brewed coffee drinks (Table 1) were lower than their corresponding LODs. The roasting and brewed coffee steps reduced the pyrethroid levels in coffee drinks. The amount of pyrethroids decreases via molecular structure breakdown (Bajwa and Sandhu, 2014) and physicochemical processes, such as evaporation, co-distillation, and thermal degradation, which may vary based on the chemical nature of the individual compound (Sharma *et al.,* 2005). No significant differences were observed in the pyrethroid concentrations ($P > 0.05$) among coffee bean preparation processes in roasted coffee beans and brewed coffee drinks.

Most pyrethroid residue levels in coffee sludge samples were lower than those in roasted coffee beans (Table 1). The cyfluthrin concentration found in coffee sludge samples from semi-washed beans was the highest (0.16 ± 0.02 μg/g). However, all pyrethroid residue concentrations in coffee sludge did not significantly differ among coffee bean preparation processes ($P > 0.05$). PCA could not be performed for pyrethroid residues as the number of detectable samples was relatively few.

3.2 Heavy metal residues in coffee samples

Most of the nine metals studied were found in green coffee beans, roasted coffee powder, brewed coffee drink, and coffee sludge, except for Cr in green coffee beans and Cd in brewed coffee drinks (only in semi-washed samples) (Table 1). In addition, the lowest and highest metal concentrations were observed in brewed coffee drinks and green coffee beans, respectively. Significant differences ($P < 0.05$) in the Cd and Fe concentrations were observed among the brewed coffee drinks.

The highest trace and toxic metal concentrations (mean ± SEM) in green coffee beans were Cu (42.56 ± 18.12 µg/g) and Pb (20.71 ± 2.52 µg/g) from semi-washed process. Copper is an inactive ingredient in some pesticides used in coffee culture. The highest concentration was found in coffee bean samples from Ethiopia, with a concentration approximately four times higher than that reported in previous studies (Şemen *et al.,* 2017; Van Cuong *et al.,* 2014). In addition to Cu, green coffee beans were contaminated with Pb at higher concentrations than those reported in other studies (Árvay *et al.,* 2019; Gure *et al,* 2017; Şemen *et al.,* 2017). However, the EU and other organizations had no established regulatory limits for Pb in green coffee beans. The metal concentration differences depend on the cultivation areas characterized by different organoleptic features and chemical compositions, which could be used as a tool for characterizing coffee variations (Ashu and Chandravanshi, 2011; Getachew and Worku, 2014). The metal residues found in green coffee bean samples are in agreement with those previously found in African coffee beans (Getachew and Worku, 2014). This likely indicates heavy metal pollution at a critical point and exceeds the international limit in many countries in this region (Ashu and Chandravanshi, 2011; Getachew and Worku, 2014; Martín *et al.,* 1998; Yabe *et al.,* 2010). This heavy metal pollution is likely due to the release of industrial wastes as well as gold mining (Yabe *et al.,* 2010). The score plot of the two principal components from the PCA analysis (Figure 1) provided an overview of the amount of heavy metal residues in samples from various regions of origin. This indicates that the potential sources of Cu, Ni, and Zn residues in green coffee bean samples in this study were America and Africa (Ashu and Chandravanshi, 2011; Getachew and Worku, 2014; Martín *et al.,* 1998).

No significant differences ($P > 0.05$) were observed in the metal residue concentrations in roasted coffee powder among the coffee bean preparation processes (Table 1). Mn concentrations (32.21 ± 2.36 µg/g) were highest in washed coffee beans, whereas Fe concentrations (32.67 ± 2.33 µg/g, 32.07 ± 2.75 µg/g) were highest in roasted powder samples of dried and semi-washed coffee beans, respectively. The concentrations of these two heavy metals corresponded to their concentrations found in the green coffee beans. Similar Mn and Fe concentrations in roasted coffee beans have been previously reported (Adler *et al.,* 2019). Metal concentrations in roasted coffee powder were higher than those in green coffee beans. It is likely that the high temperature (up to 250° C) used in the roasting process affected the chemical composition and biological activities of the green coffee beans (Adler *et al.,* 2019). Moreover, the roasting process could increase the relative concentrations by removing water and organic compounds (Getachew and Worku, 2014). Thus, increase in metal concentrations was apparent (Cruz *et al.,* 2015; Van Cuong *et al.,* 2014). In addition to coffee bean origins and qualities, coffee bean preparation processes can alter the concentrations of both trace and toxic metals (Getachew and Worku, 2014; Martín *et al.,* 1998). The Pb concentrations in all roasted coffee beans analyzed in this study were above the U.S. government policy and Brazilian permitted limits of 2.0 mg/kg and 0.5 mg/kg, respectively (Pigozzi *et al.,* 2018; USDA Foreign Agricultural Service, 2013). The levels of Pb in roasted coffee beans were greater than the Brazilian regulatory limit

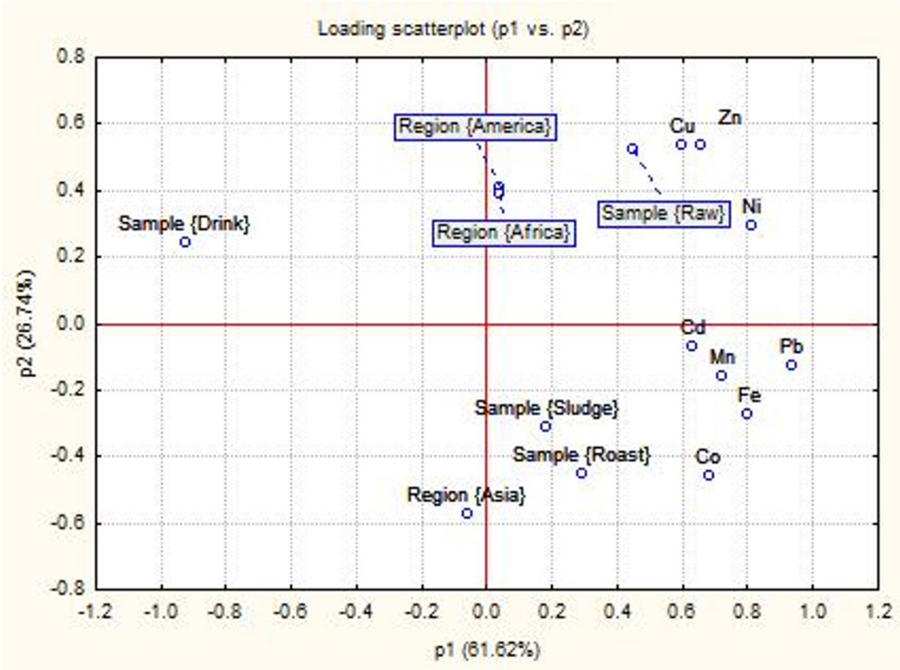

Figure 1. Factor loading after the principal component analysis of heavy metal residues in coffee bean samples

(0.5 mg/kg) and were also reported in another study (da Silva *et al.,* 2017). All metals are non-degradable soil and water contaminants; therefore, they can exist for a long time in the environment (da Silva *et al.,* 2017; Schmidt *et al.,* 2009). This can result in the accumulation of metals in the roots and grains of coffee plants (da Silva *et al.,* 2017; Schmidt *et al.,* 2009).

Statistical differences in heavy metal concentrations ($P < 0.05$) among bean processes were observed in Cd, Cu, Fe, Ni, and Pb found in brewed coffee drinks. Most metals were detected, except for Cd, in the semi-washed coffee beans. The concentrations of Mn (0.62 ± 0.09 µg/mL in washed coffee beans) and Pb (0.28 ± 0.02 µg/mL in dried coffee beans) were the highest among trace and toxic metals, respectively. The detected Mn concentrations were similar to those reported by Nędzarek *et al.* (2013) but slightly higher than those found in other studies. These differences were attributed to the wide range of geographical areas with coffee cultures (Adler *et al,* 2019; Ashu and Chandravanshi, 2011; Grembecka *et al.,* 2007).

No significant differences ($P > 0.05$) were observed in the metal concentrations in coffee sludge among bean processes. The metal with the highest concentration was Fe (39.72 ± 5.23 µg/g) in dried coffee sludge. Coffee sludge has currently been used in several ways, such as agricultural organic fertilizer, bioethanol raw materials, and biogas production (McNutt and He, 2019). If metal concentrations are similar to those found in this study, the transfer of toxic contaminants can be expected (Dadi *et al.,* 2019). Metal-contaminated coffee sludge used for these purposes is of concern. The regulations of metal residues in the coffee sludge should also be established.

Table 1. Average ± standard error of the mean/SEM (µg/g or µg/mL) of pyrethroid insecticide and metal concentrations in coffee samples from dried (D), semi-washed (SW), and washed (W) processes.

Pyrethroid insecticides/metals	Average ± SEM of pyrethroid insecticide and metal concentrations (µg/g or µg/mL)											
	Green coffee beans			Roasted coffee powder			Brew coffee drink			Coffee sludge		
	D	SW	W	D	SW	W	D	SW	W	D	SW	W
Cyfluthrin 1	0.25 ± 0.16^{a}	0.11 ± 0.04^{a}	0.04 ± 0.01^{a}	0.28 ± 0.07^{b}	0.16 ± 0.02^{ab}	0.02 ± 0.00^{a}	ND	ND	ND	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
Cyfluthrin 2	0.84 ± 0.25^{b}	0.13 ± 0.03^{a}	0.10 ± 0.02^{a}	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cyfluthrin 3	0.19 ± 0.04^{b}	0.04 ± 0.02^{a}	0.03 ± 0.01^{a}	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cyfluthrin 4	0.18 ± 0.04^{a}	0.09 ± 0.05^{a}	0.06 ± 0.02^{a}	ND	ND	ND	ND	ND	ND	ND	ND	ND
ΣCyfluthrin	1.28 ± 0.33^{b}	0.23 ± 0.11^{a}	0.14 ± 0.03^{a}	0.28 ± 0.07^{b}	0.16 ± 0.02^{ab}	0.02 ± 0.00^{a}	ND	ND	ND	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
Flumethrin	0.39 ± 0.06^{a}	0.75 ± 0.01^{b}	0.24 ± 0.04^{a}	0.06 ± 0.00^{a}	0.06 ± 0.02^{a}	0.06 ± 0.03^{a}	ND	ND	ND	0.03 ± 0.01^{a}	ND	0.02 ± 0.01^{a}
Cypermethrin CisA	0.06 ± 0.01^{b}	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cypermethrin CisB	0.02 ± 0.01^{a}	0.02 ± 0.00^{a}	0.03 ± 0.01^{a}	0.00^{a}	0.00^{a}	0.01 ± 0.00^{a}	ND	ND	ND	ND	ND	0.001 ± 0.00^{a}
Cypermethrin TransC	0.01 ± 0.00^{a}	0.01^{a}	0.01 ± 0.00^{a}	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cypermethrin TransD	0.09 ± 0.01^{ab}	0.11 ± 0.05^{b}	0.06 ± 0.01^{a}	ND	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	ND	ND	ND	ND	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}
ΣCypermethrin	0.12 ± 0.02^{a}	0.09 ± 0.04^{a}	0.08 ± 0.01^{a}	0.00^{a}	0.01 ± 0.00	0.01 ± 0.00^{a}	ND	ND	ND	ND	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
Cd	0.67 ± 0.04^{ab}	0.64 ± 0.02^{a}	0.74 ± 0.02^{b}	0.50 ± 0.03^{a}	0.56 ± 0.01^{a}	0.56 ± 0.02^{a}	0.01 ± 0.00^{b}	ND	0.01 ± 0.00^{a}	0.87 ± 0.30^{a}	0.44 ± 0.04^{a}	0.48 ± 0.02^{a}
Co	0.88 ± 0.10^{a}	1.32 ± 0.43^{a}	0.92 ± 0.11^{a}	1.87 ± 0.14^{a}	1.91 ± 0.28^{a}	1.89 ± 0.14^{a}	0.02 ± 0.01^{a}	0.01 ± 0.01^{a}	0.02 ± 0.00^{a}	1.35 ± 0.17^{a}	1.19 ± 0.16^{a}	1.45 ± 0.11^{a}
Cu	35.79 ± 16.66^{a}	42.56 ±18.12^{a}	22.00 ± 5.23^{a}	12.65 ± 2.89^{a}	12.41 ±1.69^{a}	15.19 ±1.60^{a}	0.02 ± 0.00^{b}	0.02 ± 0.01^{ab}	0.01 ± 0.00^{a}	12.03 ± 1.73^{a}	15.21 ± 3.00^{a}	14.33 ±1.54^{a}
Cr	ND	ND	ND	0.13 ± 0.00^{b}	0.97 ± 0.19^{a}	0.55 ± 0.12^{ab}	0.03 ± 0.00^{a}	0.04 ± 0.00^{a}	0.03 ± 0.00^{a}	0.20 ± 0.04^{a}	0.26 ± 0.06^{a}	0.37 ± 0.08^{a}
Fe	34.74 ± 3.78^{a}	27.76 ± 2.29^{a}	27.11 ± 0.98^{a}	32.67 ± 2.33^{b}	32.07 ± 2.75^{ab}	27.07 ± 0.45^{a}	0.15 ± 0.01^{b}	0.14 ± 0.02^{b}	0.11 ± 0.01^{a}	39.72 ± 5.23^{a}	34.97 ± 2.66^{a}	28.78 ± 0.76^{a}
Mn	20.40 ± 1.99^{ab}	19.61 ± 3.40^{a}	30.56 ± 2.39^{b}	17.70 ± 1.65^{a}	21.35 ± 4.13^{ab}	31.21 ± 2.36^{b}	0.34 ± 0.06^{a}	0.33 ± 0.04^{a}	0.62 ± 0.09^{a}	19.09 ± 1.31^{a}	20.11 ± 4.57^{a}	27.45 ± 2.07^{a}
Ni	7.67 ± 0.60^{b}	10.41 ± 0.60^{a}	9.70 ± 0.54^{ab}	4.70 ± 0.38^{a}	4.28 ± 0.53^{a}	3.71 ± 0.23^{a}	0.07 ± 0.01^{b}	0.05 ± 0.02^{ab}	0.04 ± 0.01^{a}	4.26 ± 0.47^{a}	4.02 ± 0.97^{a}	3.73 ± 0.27^{a}
Pb	20.14 ± 2.16^{a}	20.71 ± 2.52^{a}	16.92 ± 0.75^{a}	23.32 ± 0.90^{a}	23.44 ± 0.98^{a}	21.19 ± 0.54^{a}	0.28 ± 0.02^{ab}	0.26 ± 0.02^{a}	0.31 ± 0.01^{b}	18.02 ± 0.67^{a}	18.02 ± 0.43^{a}	17.16 ± 0.40^{a}
Zn	20.84 ± 7.19^{a}	30.13 ± 11.61^{a}	15.59 ± 3.46^{a}	9.27 ± 1.65^{a}	7.66 ± 0.92^{a}	8.64 ± 0.96^{a}	0.07 ± 0.01^{a}	0.07 ± 0.01^{a}	0.08 ± 0.01^{a}	8.50 ± 0.97^{a}	9.84 ± 1.75^{a}	7.49 ± 0.93^{a}

Averages followed by different letters indicated significant ($P < 0.05$) differences among coffee bean processes.
Nd : Non detectable (concentrations < LOD)

3.3 Determination of processing factors

No significant difference (P > 0.05) was observed in the PF in pyrethroid residues among bean processes (Table 2). The significant differences (P < 0.05) in PF, however, existed in most heavy metals except for Cu and Zn detected in the semi-washed coffee beans. The PF of pyrethroids from all coffee bean preparation processes was less than 1. This indicates that coffee roasting and brewing decreased the amount of pyrethroid residues. These results are in agreement with those of other studies, which reported that the PFs decreased after food processing (Bonnechere *et al.,* 2012; Mekonen *et al.,* 2015). The roasting process plays the most important role in the reduction of pyrethroid concentrations. However, the PF in pyrethroids that were not detected in the samples was not calculated. By contrast, higher concentrations of most metals were observed after roasting, as indicated by PF greater than 1 (Table 2). The increase in concentrations were likely due to the evaporation of water and/or combustible mass in the coffee grain, which concentrates metals in the coffee beans (Adler *et al.,* 2019). The brewed coffee drinks showed the opposite processing factor (PF of < 1), with percentage reductions greater than 96%. This indicated that coffee brewing resulted in the reduction of all metal concentrations and was likely due to the dilution effect of their weight reduction.

3.4 Human health risk assessment

None of the pyrethroid pesticides were detectable in brewed coffee drinks. By contrast, all metals were found in brewed coffee drinks, but the concentrations were lower than the reference ADIs or RfDs (HRI < 1). The EDIs indicated that the concentrations of toxic metals (Cd, Cr, Ni, and Pb) and pyrethroid insecticides (cyfluthrin, cypermethrin, and flumethrin) in brewed coffee drinks by normal drinkers and 97.5th percentile drinkers of the Thai population were within the acceptable limits. Among the toxic metals, the concentration of Pb found in brewed coffee drinks (0.31 μg/mL) was the highest. Its highest EDIs in Thai individuals with normal consumption (216.06 mL/day) and in the 97.5th percentile (330 mL/day) of Thai coffee drinkers (National Bureau of Agricultural Commodity and Food Standards, 2016) were 0.97 mg/kg BW/day (washed) and 1.48 mg/kg BW/day (washed), respectively. In addition, all calculated HRIs that were less than 1 (Table 3) indicated that the human consumption of all studied samples was relatively safe. Trace metals such as Co, Cu, Fe, Mn, Ni, and Zn are cofactors of a large number of enzymes. Their trace but adequate amounts are essential for normal body functions (Cu, 0.9 mg/day; Fe, 8 – 18 mg/day; Mn, 1.8 – 2.3 mg/day; Ni, 0.5 mg/day; and Zn, 8 – 11 mg/day) (Nogaim *et al.,* 2014). However, excess amounts of these trace metals can cause adverse human health effects. The estimated consumption of these metals in brewed coffee drinks from this study was higher than the normal human requirement but still safe for consumption. A few studies showed no health risk effects of mineral intake following coffee consumption, which is similar to the findings in this study (Adler *et al.,* 2019; Şemen *et al.,* 2017). The THQ values were calculated and used to assess the human health risks for long-time consumers (Table 3). In this study, the THQ of all investigated analytes was less than 1. Thus, the consumption of brewed coffee drinks from all studied coffee bean samples is unlikely to have adverse effects on human health.

4. Conclusion

Coffee is among the most popular beverages in all countries. Insecticides and metals introduced during coffee cultivation could result in the contamination of coffee beans. These contaminants could be reduced during coffee bean processing, including drying, semi-washing, and washing. In this study, three pyrethroid insecticides and nine metals were determined in coffee samples: green coffee beans, roasted coffee powder, brewed coffee drink, and coffee sludge. Coffee roasting reduced the concentrations of pyrethroid insecticides and two heavy metals (Cd and Ni), as indicated by the PF value, which was less than 1. In addition, coffee brewing could reduce the concentrations of both pyrethroid insecticides and metals with similar values. Based on the normal and 97.5th percentile consumption of the Thai population, it can be concluded that the consumption of brewed coffee drinks is unlikely to have adverse effects on human health. The use of coffee by-products (coffee sludge) as fertilizers or raw biogas materials should be of concern because they may contain toxic contaminants.

Table 2. Average processing factor (PF) ± standard error of the mean/SEM and average percentage reduction (%) of pyrethroid insecticides and metals in coffee samples from dried, semi-washed and washed processes.

Pyrethroid / metals residues	Average processing factor (PF) ± SEM and average percentage reduction in parenthesis (%)								
	Dried process			Semi-washed process			Washed process		
	Roasted beans	Brew coffee drink	Coffee sludge	Roasted beans	Brew coffee drink	Coffeee sludge	Roasted beans	Brew coffee drink	Coffee sludge
Cyfluthrin 1	0.56 ± 0.50 (43.77%)	NA	0.01 ± 0.01 (99.06%)	0.00 ± 0.00 (100.00%)	NA	0.00 ± 0.00 (100.00%)	0.01 ± 0.01 (99.19%)	NA	0.12 ± 0.08 (88.08%)
Cyfluthrin 2	0.00 ± 0.00 (100.00%)	NA	0.00 ± 0.00 (100.00%)	0.00 ± 0.00 (100.00%)	NA	0.00 ± 0.00 (100.00%)	0.00 ± 0.00 (100.00%)	NA	0.00 ± 0.00 (100.00%)
Cyfluthrin 3	0.00 ± 0.00 (100.00%)	NA	0.00 ± 0.00 (100.00%)	0.00 ± 0.00 (100.00%)	NA	0.00 ± 0.00 (100.00%)	0.00 ± 0.00 (100.00%)	NA	0.00 ± 0.00 (100.00%)
Cyfluthrin 4	0.00 ± 0.00 (100.00%)	NA	0.00 ± 0.00 (100.00%)	0.00 ± 0.00 (100.00%)	NA	0.00 ± 0.00 (100.00%)	0.00 ± 0.00 (100.00%)	NA	0.00 ± 0.00 (100.00%)
ΣCyfluthrin	0.59 ± 0.50 (40.83%)	NA	0.01 ± 0.00 (99.81%)	0.28 ± 0.21 (72.21%)	NA	0.01 ± 0.01 (98.81%)	0.02 ± 0.01 (98.44%)	NA	0.06 ± 0.03 (94.50%)
Flumethrin	0.02 ± 0.01 (98.05%)	NA	0.04 ± 0.02 (96.00%)	0.07 ± 0.06 (93.16%)	NA	0.00 ± 0.00 (100.00%)	0.27 ± 0.09 (73.26%)	NA	0.01 ± 0.00 (99.14%)
Cypermethrin CisA	0.00 ± 0.00 (100.00%)	NA	0.00 ± 0.00 (100.00%)	0.00 ± 0.00 (100.00%)	NA	0.00 ± 0.00 (100.00%)	0.00 ± 0.00 (100.00%)	NA	0.00 ± 0.00 (100.00%)
Cypermethrin CisB	0.04 ± 0.00 (95.64%)	NA	0.00 ± 0.00 (100.00%)	0.05 ± 0.04 (94.66%)	NA	0.00 ± 0.00 (100.00%)	0.10 ± 0.04 (89.62%)	NA	0.01 ± 0.01 (98.68%)
Cypermethrin TransC	0.00 ± 0.00 (100.00%)	NA	0.00 ± 0.00 (100.00%)	0.00 ± 0.00 (100.00%)	NA	0.00 ± 0.00 (100.00%)	0.00 ± 0.00 (100.00%)	NA	0.00 ± 0.00 (100.00%)
Cypermethrin TransD	0.00 ± 0.00 (100.00%)	NA	0.00 ± 0.00 (100.00%)	0.12 ± 0.05 (88.39%)	NA	0.00 ± 0.00 (100.00%)	0.05 ± 0.03 (95.14%)	NA	0.01 ± 0.00 (98.76%)

Averages followed by different letters indicated significant ($P < 0.05$) differences among coffee bean processes.
NA (not available): the processing factor was not calculated due to pesticide or metal residues were not detected.

Table 2. Average processing factor (PF) ± standard error of the mean/SEM and average percentage reduction (%) of pyrethroid insecticides and metals in coffee samples from dried, semi-washed and washed processes. (Cont.)

Pyrethroid / metals residues	Average processing factor (PF) ± SEM and average percentage reduction in parenthesis (%)								
	Dried process			Semi-washed process			Washed process		
	Roasted beans	Brew coffee drink	Coffee sludge	Roasted beans	Brew coffee drink	Coffeee sludge	Roasted beans	Brew coffee drink	Coffee sludge
ΣCypermethrin	0.01± 0.01 (99.85%)	NA	0.00 ± 0.00 (100.00%)	0.11 ± 0.02 (89.27)	NA	0.00 ± 0.00 (100.00%)	0.97 ± 0.03 (90.26%)	NA	0.05 ± 0.03 (95.32%)
Cd	0.79 ± 0.06^{ab} (21.54%)	0.01 ± 0.00^{a} (98.99%)	1.37 ± 0.47^{b} (-36.89%)	0.88 ± 0.03 (12.09%)	NA	0.69 ± 0.05 (30.89%)	0.77 ± 0.03^{c} (22.77%)	0.00 ± 0.00^{a} (99.78%)	0.65 ± 0.03^{b} (35.26%)
Co	2.36 ± 0.35^{b} (-135.67)	0.02 ± 0.00^{a} (97.80%)	1.65 ± 0.25^{b} (-65.08%)	2.21 ± 1.11^{b} (-121.42%)	0.01 ± 0.00^{a} (99.74%)	1.39 ± 0.65^{b} (-39.25%)	4.57 ± 1.04^{b} (-356.99%)	0.04 ± 0.00^{a} (96.230%)	3.32 ± 0.87^{b} (-232.00%)
Cr	NA	NA	NA	NA	NA	NA	NA	NA	NA
Cu	1.04 ± 0.23^{b} (-4.408%)	0.00 ± 0.00^{a} (99.79%)	1.08 ± 0.21^{b} (-7.76%)	0.68 ± 0.27^{a} (32.11%)	0.01 ± 0.00^{a} (99.92%)	1.21 ± 0.72^{a} (-20.92%)	1.19 ± 0.15^{b} (-18.59%)	0.01 ± 0.00^{a} (99.88%)	1.11 ± 0.14^{b} (-10.53%)
Fe	1.03 ± 0.08^{b} (-3.27%)	0.01 ± 0.00^{a} (99.54%)	1.25 ± 0.15^{b} (-25.34%)	1.16 ± 0.06^{b} (-16.20%)	0.01 ± 0.00^{a} (99.50%)	1.27 ± 0.06^{b} (-26.88%)	1.02 ± 0.03^{b} (-2.16%)	0.01 ± 0.00^{a} (99.58%)	1.10 ± 0.04^{b} (-9.06%)
Mn	0.88 ± 0.03^{b} (11.69%)	0.02 ± 0.00^{a} (98.45%)	0.98 ± 0.05^{c} (2.16%)	1.11 ± 0.10^{b} (-10.85%)	0.02 ± 0.00^{a} (98.23%)	1.03 ± 0.11^{b} (-2.97%)	1.05 ± 0.05^{b} (-4.68)	0.02 ± 0.00^{a} (98.06%)	0.93 ± 0.06^{b} (7.07%)
Ni	0.97 ± 0.39^{b} (2.64%)	0.02 ± 0.01^{a} (98.24%)	0.86 ± 0.33^{ab} (14.55%)	0.41 ± 0.03^{b} (59.29%)	0.01 ± 0.00^{a} (99.51%)	0.38 ± 0.09^{b} (61.56%)	0.39 ± 0.03^{b} (60.90%)	0.01 ± 0.00^{a} (99.60%)	0.41 ± 0.03^{b} (59.55%)
Pb	1.28 ± 0.11^{c} (-28.42%)	0.02 ± 0.00^{a} (98.42%)	1.00 ± 0.09^{b} (0.20%)	1.20 ± 0.15^{b} (-20.35%)	0.013 ± 0.00^{a} (98.67%)	0.91 ± 0.09^{b} (8.80%)	1.31 ± 0.06^{c} (-31.33%)	0.03 ± 0.00^{a} (98.06%)	1.06 ± 0.04^{b} (-5.75%)
Zn	0.99 ± 0.22^{b} (1.43%)	0.01 ± 0.00^{a} (99.35%)	0.86 ± 0.14^{b} (13.88%)	0.50 ± 0.19^{a} (49.56%)	0.01 ± 0.00^{a} (99.48%)	0.77 ± 0.37^{a} (23.35%)	1.23 ± 0.39^{b} (-22.85%)	0.01 ± 0.00^{a} (99.05%)	1.10 ± 0.36^{b} (-9.70%)

Averages followed by different letters indicated significant ($P < 0.05$) differences among coffee bean processes.
NA (not available): the processing factor was not calculated due to pesticide or metal residues were not detected.

Table 3. Estimated daily intake (EDI) of exposure levels to pesticide (µg/kg BW/day) and metal (µg/kg BW/day) residues detected in brew coffee drink,health risk index (HRI) and average target hazard quotient (THQ) based on normal consumption and the 97[th] percentile consumption of Thai population.

Pyrethroid / metals residues	Normal consumption									The 97.5[th] percentile consumption								
	Dried process			Semi-washed process			Washed process			Dried process			Semi-washed process			Washed process		
	EDI	HRI	THQ	EDI	HRI	THQ	EDI	HRI	THQ	EDI	HRI	THQ	EDI	HRI	THQ	EDI	HRI	THQ
ΣCyfluthrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ΣCypermethrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Flumethrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cd	0.02	0.02	0.02	ND	ND	ND	0.01	0.00	0.01	0.03	0.03	0.03	ND	ND	ND	0.01	0.01	0.01
Co	0.06	NA	NA	0.04	NA	NA	0.05	NA	NA	0.09	NA	NA	0.06	NA	NA	0.08	NA	NA
Cr	0.08	0.03	0.03	0.11	0.04	0.05	0.09	0.03	0.04	0.12	0.04	0.04	0.17	0.06	0.06	0.13	0.04	0.04
Cu	0.07	0.00	< 0.01	0.05	0.00	< 0.01	0.05	0.00	< 0.01	0.10	0.00	< 0.01	0.08	0.00	< 0.01	0.07	0.00	< 0.01
Fe	0.45	0.00	< 0.01	0.43	0.00	< 0.01	0.34	0.00	< 0.01	0.69	0.00	0.01	0.66	0.00	0.01	0.52	0.00	0.01
Mn	1.04	0.01	0.01	1.02	0.01	0.01	1.91	0.01	0.02	1.58	0.01	0.01	1.56	0.01	0.01	2.91	0.02	0.02
Ni	0.22	0.01	0.01	0.17	0.01	0.01	0.12	0.01	0.01	0.34	0.02	0.02	0.25	0.01	0.01	0.18	0.01	0.01
Pb	0.87	0.04	0.04	0.79	0.03	0.04	0.97	0.04	0.05	1.33	0.05	0.05	1.21	0.05	0.05	1.48	0.06	0.06
Zn	0.22	0.00	< 0.01	0.22	0.00	< 0.01	0.24	0.00	< 0.01	0.34	0.00	0.01	0.33	0.00	0.01	0.36	0.00	0.01

NA : not available therefore the EDI or THQ was not calculated due to no reports or references of acceptable daily intake.
ND : Not detectable.

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