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Agrochemical Residues in Soil, Water, Chicken's Blood and Worker's Urine Samples in Rose Farms, Tak Province, Thailand

ABSTRACT

The agrochemical overuse, especially that of paraquat, cypermethrin and carbendazim, has been observed in Thailand. Residuals do not only contaminate the environment and agricultural products, but also other living organisms related to the farming. With less attention being paid to the agrochemical residues in the environment, this study aimed to quantify agrochemical residues in soil, water, chicken's blood and worker's urine samples across different periods of cultivation among nine rose farms in Tak Province, Thailand were selected as study sites. Various agrochemicals were used throughout the year; particular attention was paid to paraquat, cypermethrin and carbendazim. Soil and water samples represented the environment, chicken's blood represented animal subjects, and urine samples of rose farmers represented human subjects. The study revealed the existence of those three agrochemical residues in both soil and water samples. It was only in soil samples that paraquat and cypermethrin concentrations were higher than the maximum allowable concentration (47.24 mg kg^{-1} and 0.24 mg kg^{-1}, respectively). Residues of cypermethrin and carbendazim were found only in chicken's blood (0.0280 µg L^{-1} and 0.0750 - 0.1640 µg L^{-1}, respectively). Since rose farmers were well equipped with personal protective equipment when applying the pesticides, paraquat, cypermethrin and carbendazim quantifications in their urine samples were all below the detectable limits of the instruments used. Further studies such as better methods for residue analysis and a larger sample size are needed. More reliable quantifications may possibly result in assessing the relationship between agrochemical residues among environmental and living organisms.

Keywords: *agrochemical residues, paraquat, cypermethrin, carbendazim, soil, water, chicken's blood, human urine samples*

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INTRODUCTION

Thailand is known as an agricultural country producing rice, vegetables, fruits, rubber and flowers from past to present. It is also known as 'kitchen of the world' (*BOI 2018; WSJ 2022*). While the perception of the world is focused on 'green food', the Thai agricultural industry is still using the traditional platform. High yield of agricultural products is expected and agrochemicals particularly pesticides and fertilizers are used to improve and protect crops. Numerous studies in Thailand showed that the more agrochemicals were used, the less they were controlled; hence they contaminated the environment (*Poramacom 2001; Prathumintr 2006; Sricharoen 2007; Jinjutha 2010; Purisumban 2011; Unprasert 2011; Tirado et al. 2016*). Moreover, some studies indicated that the high number of imported agrochemicals had been rising every year due to the lack of knowledge, high demand of crop production and the government's policies. Data from the Office of Agricultural Economics revealed that in 2017, up to 198,317 t of agrochemicals were imported (accounted for 20% growth rate compared to the year 2011) (*OAE 2017*). The excessive agrochemicals are still the major problem at present and may destroy Thai agricultural industry in the near future, if policy makers and Thai farmers do not give proper attention.

Agrochemical residues in the environment such as soil, water and agricultural products, as well as their health impact are generally recognized (*Singh et al. 2020; Manda et al. 2020; Sangsirimongkolying et al. 2015; Pibul 2021*). The harmful effects can be reduced by washing the products before consumption or delaying the harvest. Knowledge on the persistent excessive agrochemicals in the environment and living organisms may urge the major stakeholders in the agriculture industry to pay attention to its issue.

Tak Province has been ranked in the top ten provinces of high crop production (*OAE 2010*). Currently, Phop Phra District in Tak Province has 560 ha for cultivating roses due to its appropriate climatic condition and terrain (*Jierwiriyapant et al. 2000; Sricharoen 2007*). Another property is a group of soil in Phop Phra District that have a good water drainage and good water surface runoff that are the group of soil 29, 46, and 62 (*Sricharoen 2007; LDD 2000*). Due to the high volume of rose demand, the high agrochemicals are used, and the excessive agrochemicals are accumulated in the environment (*Prathumintr 2006; Zhang et al. 2018*). In rose farms, Paraquat, Cypermethrin and Carbendazim were used to increase productivity as part of the cultural practices of managing crop pests and diseases.

Paraquat, a kind of herbicide, is categorized in the group of pyridines and quaternary ammonium compounds. It is a defoliant and desiccant agent used for both pre- and post-emergence of pests. It is hydrolytically stable at pH 5, 7 and 9 after 30 d of application at 25 and 40°C (*Watts 2010*). It is quickly absorbed through plant foliage and remains in the leaves while the average field half-life for paraquat in the soil has been estimated at 1,000 days. Paraquat in the soil does not affect plant emergence or growth. Its long persistence is due to very limited microbial degradation and the strong sorption of paraquat to clay particles. In human urine, paraquat is rapidly but incompletely absorbed and then largely eliminated unchanged in urine within 12-24 hrs. Four hours of inhalation of paraquat can cause skin and eye irritation in rabbits and skin sensitization in guinea pigs. In high acute exposure, it can lead to convulsions, and death by respiratory failure. If swallowed, burning of the mouth and throat often occurs, followed by gastrointestinal tract irritation, resulting in abdominal pain, loss of appetite, nausea, vomiting, and diarrhea. Repeated exposures to paraquat may cause skin irritation, sensitization or ulceration on contact. After exposure for two years at high dose, paraquat can cause lung problems (*ETOXNET 1993; European Commission 2003c*).

Cypermethrin, a kind of insecticide, is a member of pyrethroids. These compounds are degraded in soil and have no detectable effects on soil microflora (*Indratin 2019*). Its hydrolytic stability (DT_{50}) is 923 -1,302 d at 25°C, pH 3; 136-221 d at pH 7; 5-21.2 d at pH 8 and 23-28 mins at pH 11. Its half-life in soil is 60 days (*European Commission 2003b*). Cypermethrin also photodegrades rapidly on soil surfaces to many by products, with half-lives of 8-16 d. It is fairly immobile in soil due to its strong affinity to bind to the organic matter. In water, it binds to the organic substances and persists over 50-100 d in the river. After dermal exposure, acute toxicity is hypersensitivity; ingestion tract nausea then diarrhea. Chronic toxicity of cypermethrin happened to the skin is burning, numbness and itching.

Carbendazim methyl benzimidazol-2-yl-carbamate) is a type of fungicide with hydrolytic stability of >350 days at 22°C with pH 5 and > 350 d at pH 7 (*European Commission 2003a; FAO 2009*). Carbendazim can persist in sterilized sand for up to 350 d but up to 180 d in non-sterilized soil. Carbendazim is strongly adsorbed to soil organic matter and remains in the soil for up to three years. Under sterile conditions, carbendazim was found to be stable at pH 5 and 7 for 30 d and 9 d at pH 9. In humans, Carbendazim is rapidly excreted in the urine and feces. There is insufficient information about the toxicity of carbendazim in humans.

The study aims to quantify paraquat, cypermethrin and carbendazim residues in soil, water, chicken's blood and worker's urine samples during different periods of cultivation to determine possible overuse of identified agrochemicals in the rose farms and exceeding its allowable concentrations.

MATERIALS AND METHODS

Study area and study period

Rose farms in Phop Phra District in Tak Province, Northern Thailand (**Figure 1**) were of particular interest since their rose plantation requires a number fungicides,

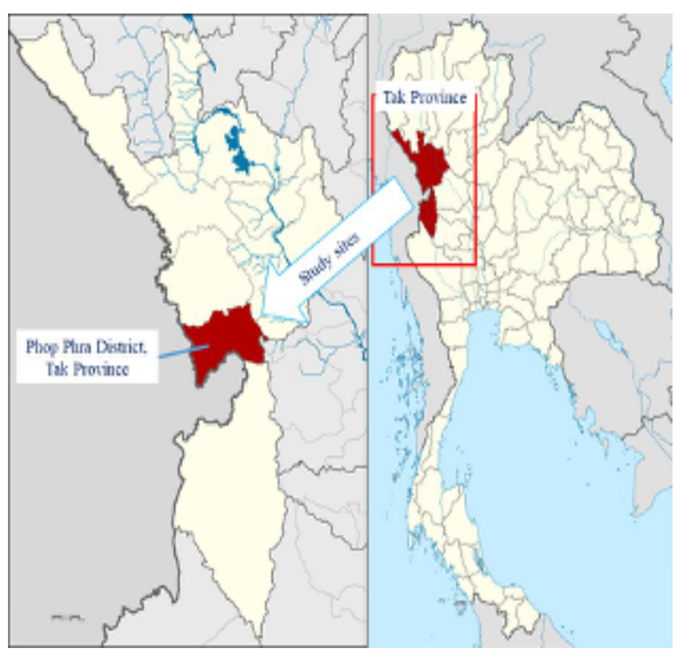

Figure 1. Phop Phra District, Tak Province.

and fertilizers. Agrochemicals are highly used of agrochemicals, such as insecticides, pesticides, on a daily basis in order to prevent roses from pests. The popular herbicide, insecticide, and fungicide used in Rose farms are paraquat, cypermethrin, and carbendazim, respectively. Of the 20 farms in Phop Phra District, nine were selected as a tracer setting based on two criteria: at least one pond in the rose farm, and workers who sprayed pesticides are residing in the farm.

Sample collections (soil, water, chicken blood and pesticide applicators' urine samples) were collected twice, during the dry season and rainy season, between December 2015 to September 2016. Since pesticide was applied every day, sample collections were performed any day with permission from the farm owners.

Sample materials

Soil collection and analytical method

Ten soil samples were collected in each farm following the methods of *Sava* (*1994*) and *Tadeo* (*2008*). Samples were collected 15 cm deep from the surface near the rose plots using the V shape method. The samples were collated in a bucket making it approximately 1 kg to represent that particular farm. Soil characteristics, pH and moisture were determined.

The QuEChERS Technique was used to prepare soil samples before the determination of the paraquat, cypermethrin and carbendazim residues. The 25 g of soil was added with deionized water, iso-octane and sulfuric acid then refluxed for five hours. The soil sample was rinsed with deionized water and adjusted to pH 9 with 10N sodium hydroxide. The mixture was later filtered through filter paper No.1, cleaned up through SPE-silica 500 mg and finally eluted with 10 mL of 8N HCl :MeOH 9:1.

Soil samples undergoing QuEChERS Technique were subject to residue quantification using Liquid Chromatograph-Mass Spectrometer (LC-MS, HP1100 Modular LC-MS system, Agilent Technology, Palo Alto, CA, USA).

Water collection and analytical method

At each farm, water samples were collected from water sources in and nearby the farms following the method of *Tadeo* (*2008*). The distances between wells to plot areas were 50-200 m. About 1 L of water sample (composite sample) was kept in a plastic bottle at 4°C before analysis. The pH at the time of collection was recorded.

The QuEChERS Technique was used to prepare water samples before the determination of the paraquat, cypermethrin and carbendazim residues. A 200 mL of water sample was loaded in resin SPE cartridges by using a peristaltic pump. The cartridge was rinsed with 5 mL of methanol-deionized water (50:50) and air dried for 1 min. It was then eluted by using 5 mL of 1M ammonium chloride in methanol-deionized water (50:50) and the sample extracts were filtered through a 0.45 μm nylon filter. The filtered water extract was then analyzed also using the LC-MS..

Collection of chicken's blood and analytical method

Blood samples were collected from chickens that were fed in rose farms by Animal Husbandry employed by the farm. One to three mL of blood was collected from the vein under the wing of the chicken. At each time of sample collection, there were 15 chicken's blood samples collected from nine farms in this study. Chicken blood was kept in an EDTA tube and kept in 4°C before analysis. In-house methods of Siriraj Poison Control Center, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand, were employed to determine the residues of paraquat, cypermethrin and carbendazim.

Collection of urine in workers and analytical method

Urine samples were collected from the workers who sprayed pesticides and/or dug the soil in the farms. Before urine sample collection, workers were interviewed for approximately 30 min. Issues covered by the interview included duration of work in rose farm, the frequency of spray agrochemical per day, knowledge on pesticides usage, knowledge on personal protection equipment, knowledge, and perception on impacts of pesticides on health. The workers were instructed on how to collect the urine. Thirty mL of mid-stream urine was collected from each worker. There were nine urine samples collected at each time of urine sample collection. Urine samples were kept in a plastic bottle and kept in 4°C before analysis. In-house methods of the toxicology department of Siriraj Poison Control Center, Faculty of Medicine Siriraj Hospital, Mahidol University Thailand, were employed to determine the residues of paraquat, cypermethrin and carbendazim.

Protocol of the study in human subjects was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Thailand (Approval No. MUTM 2015-072-01).

Statistical analysis

Concentrations of agrochemical residues in soil samples, water samples, chicken bloods, and worker urine samples were reported as descriptive data. Comparison between quantifications in the rainy and dry season was determined using pair t-test at p-value < 0.05.

RESULTS AND DISCUSSION

Characteristics of selected rose farms

Based on the criteria mentioned earlier, nine rose farms were selected. In each farm, there were 5-15 workers but only one or two were responsible for spraying pesticides. The other duties were cutting flowers, shoveling, fertilizing, and watering flowers. All of the farms except Farm number 2 has less than 100,000 m^2 land area (**Table 1**).

The average outdoor air temperatures at the study sites were 20-25°C during daytime and approximately 20°C at night. In the dry season, ground water and/or water from the ponds were used for watering while watering in the rainy season depended mostly on the rain. Some rose farms collected rainwater using big buckets and/or digging a pond to keep water for dry season usage.

Table 1. Total area of rose cultivation and distance from water resource to the rose plot.

Farm	Area of rose farm (m^2)	Distance from water source to rose plot (m)
1	80,006.35	130.48
2	112,016.99	101.72
3	71,993.58	89.29
4	75,190.59	57.48
5	75,190.59	148.16
6	80,006.35	65.96
7	76,121.37	164.22
8	95,991.43	140.81
9	80,006.35	134.41

Rose farmers usually watered the rose twice a day: early in the morning and another one in the evening. On some days when the soil was dry, watering was increased to three times per day. There was not much difference in the period of sunlight between the rainy season and dry season i.e., at least five hours per day.

The pH and moisture of the soil were determined during the soil sample collection (**Table 2**). The soil group was based on the characterization by the Land Development Department (*LDD 2014*).

Observation has shown that there were two farms with soil group 29, six with soil group 46 and one with soil group 62. Soil moisture in the rainy season was apparently higher than that of dry season except in Farm 3 where soil moisture in the dry season was twice as much that as that of the rainy season. It was noted that the soil collection at Farm 3 in dry season was performed after the worker watered the plants. For soil pH, there seemed to be no differences in soil pH regardless of the soil group and the season. The above characteristics supported the study of *Sinsorn* (*2014*) who reported that this area was appropriate for rose cultivation.

Analyses of agrochemical residues in soil samples

Paraquat was found in all rose farms and in both seasons while the concentrations of carbendazim and cypermethrin were sometimes lower than the detectable limit of the instrument used. When comparing the concentrations of agrochemical residues in soil samples collected in rainy season with those in the dry season, comparison of carbendazim and cypermethrin residues in rainy season and dry season could not be performed

Table 2. Characterization, moisture and pH of soil samples collected in rainy and dry seasons.

Farm	Soil group*	Rainy season		Dry season	
		Soil moisture (g 100 g^{-1} soil)	Soil pH	Soil moisture (g 100 g^{-1} soil)	Soil pH
1	29	7.19	7.50	2.58	8.00
2	62	17.45*	7.80	2.94	7.60
3	46	8.49	8.00	17.63*	7.80
4	46	3.06	7.90	2.28	8.00
5	46	2.87	7.50	2.60	7.80
6	46	2.18	7.60	2.00	7.80
7	46	3.50	7.80	3.06	7.80
8	46	3.51	7.80	1.91	8.00
9	29	6.48	8.00	2.72	8.20

Note: Soil samples were collected after watering; *Characterization of soil followed *LLD* (*2014*).

due to the lack of their concentrations in some of the study periods while those of paraquat were significantly different (p = 0.012).

Most agrochemical residues, paraquat, cypermethrin and carbendazim were found in soil samples (**Table 3**). This was consistent with the responses from the workers who claimed that they were spraying different agrochemicals every day. Agrochemical application is dependent on the weather condition. For instance, if the weather was high in moisture and cool in the early morning, the farm owner would prepare carbendazim for spraying first, followed by cypermethrin to kill insects later of the day and paraquat was sprayed during afternoon or evening after the workers have cut flowers and shoveled the soil at rose plot. Especially in the rainy season, spraying was scheduled twice a day.

During the rainy season, cypermethrin and carbendazim were found to be lower than paraquat. This could be due to the fact that cypermethrin and carbendazim could not bind with soil texture (*EXTOXNET 1993; Cole 2002; Nollet 2010*) and were decomposed by bacteria in soil (*European Commission 2003a; European Commission 2003b; Krieger 2009*). It could also be due to competitive adsorption among agrochemicals. Appropriate condition for retaining carbendazim in the soil was acidic (*Lewandowska 2010*). Therefore, carbendazim residue was not found in soil samples due to its alkaline condition. Cypermethrin is a non-ionic compound with a tendency to be washed-off from the soil and can be subjected to photolysis by ultraviolet radiation (*Fortin 2003; EPA 2006*). Paraquatwas mostly found in all farms for both seasons due to the fact that it persists in clay soil (*Madeley 2006; LDD 2014*) or Soil Type 46. *Amondham et al.* (*2006*) reported that only 0.04-0.17% of paraquat was released from clay. Comparing the amount of pesticide load of 7,000-8,000 L-time^{-1}, one can assume that excessive pesticides caused the high residues of agrochemicals in soil.

For the pH of soil (**Table 2**), the pH of soil group 29 usually ranges from 6.5-8.0 (*LDD 2014*), while those of soil groups 46 and 62 is mainly acidic (4.5-6.5). However, pH of soil in this study was rather alkaline, which could be the result of fertilizers and agrochemicals used. These chemicals had an effect on soil pH (*Prathumintr 2006; Sricharoen 2007; Tirado 2016*). Cypermethrin was not detected in some soil samples (i.e., detection limit is 0.01 mg kg^{-1}).

Both paraquat and cypermethrin concentrations were higher than the maximum acceptable concentration of 0.01 mg kg^{-1} (*Watson 1998; Madeley 2006*). However, the maximum allowable concentration of carbendazim has not been specified (*EPA 2005*).

Analyses of agrochemical residues in water samples

There was not much difference in water pH in both seasons. The pH ranged from 7.50 to 8.30 and it was noted that water samples from Farm 7 had the most alkalinity in both seasons which was in accordance with *Multhongnoi* (*2008)* who showed the average is pH 7.3-8.0 natural water in Tak Province (**Table 4**).

Analyses of agrochemical residues revealed the presence of paraquat and carbendazim in some of the water samples while cypermethrin was absent or lower than detectable limit in all water samples (**Table 5**).

Due to the failure in detecting agrochemicals in most the water samples, comparing their concentration in rainy season and dry seasons was impossible.

Table 3. Agrochemical residues in soil samples during the rainy and dry season.

Farm	Rainy season			Dry season		
	Paraquat (mg kg^{-1})	Cypermethrin (mg kg^{-1})	Carbendazim (mg kg^{-1})	Paraquat (mg kg^{-1})	Cypermethrin (mg kg^{-1})	Carbendazim (mg kg^{-1})
1	12.48	0.24	ND	24.94	0.03	0.03
2	4.53	ND	0.02	30.53	ND	2.68
3	4.32	ND	0.17	11.54	ND	0.17
4	9.80	0.02	ND	7.36	0.04	0.04
5	8.13	0.01	0.01	19.36	ND	0.02
6	7.70	0.02	0.02	21.21	ND	0.01
7	2.58	0.05	0.02	34.80	0.01	0.61
8	2.18	0.06	ND	47.24	0.01	0.02
9	6.42	ND	ND	9.22	0.22	0.03

ND: Lower than detectable limit (for LC-MS) of 0.01 mg kg^{-1}

Table 4. pH of water sample.

Farm	Water pH	
	Rainy season	Dry season
1	7.80	7.90
2	7.80	7.80
3	7.80	7.90
4	7.50	7.50
5	7.80	7.90
6	7.80	7.80
7	8.20	8.30
8	7.70	7.80
9	7.50	7.50

The concentration of agrochemicals in water was mostly below detection limit both in the rainy season and dry season (**Table 5**). This could be the result of the distance between the spraying area and water sources. Besides, water sources were situated upwind from the plot and sometimes there was a natural barrier such as a fruit orchard.

Properties of the agrochemicals may play a part in this situation. Paraquat can strongly bind to the soil texture better than cypermethrin and carbendazim (*European Commission 2003a, Esuropean Commission 2003b; EPA 2006; Krieger 2009*). *Madeley* (*2006*) also reported that paraquat can strongly bind with the sediment making paraquat hardly show up in surface water. In addition, paraquat is unstable in alkaline water and can be decomposed by ultraviolet light (*European Commission 2003; Krieger 2009*).

A reason for the absence of carbendazim and cypermethrin in water samples could be due to their half-life of 0.7 d in alkaline water or their stability in water samples. The water samples in this study were kept in 4°C for 3-5 d before analysis due to the transportation problems. Therefore, carbendazim may be decomposed by bacteria (*European Commission 2003a; EPA 2006; Krieger, 2009*). On the other hand, cypermethrin is stable in acidic water, usually pH 4 (*Heitzman 1997*) but the pH of the water samples ranged from 7.50-8.20 (**Table 4**). This could be the reason for the absence of cypermethrin in this study as well.

Maximum concentration of 0.08 mg L^{-1} of paraquat is obtained in the rainy season while the lowest of 0.01 mg L^{-1} was reported in dry season. Such concentration of paraquat was considered high as its maximum allowable concentration in water is set at 0.001 mg L^{-1} (*EPA 1997; Marrs 2003; Madeley 2006*). As for the concentration of agrochemical residues in water samples, cypermethrin was not detected in all of the water samples even though the maximum allowable concentration has been set at 2 mg L^{-1} (*Cole 2002; Woollen et al. 1992*). Concentration of carbendazim also varied, with some not detectable to the highest of 0.09 mg L^{-1} in dry season while the maximum allowable concentration was set at 0.1 mg L^{-1}.

Analyses of agrochemical residues in chicken's blood samples

The residues of carbendazim and cypermethrin were found in chickens of only two farms while paraquat residue quantification was below the detection limits of the instruments used (**Table 6**).

The chickens were fed on an open system near the rose farm. This study found the residues of carbendazim and cypermethrin in some chicken blood samples. In Farm 3, the residue of cypermethrin was not found in soil samples but in chicken blood. This may be due to the fact that the chickens were exposed directly to cypermethrin or from other sources rather than the soil. This is similar to the results from Farms 3 and 5 where carbendazim

Table 5. Agrochemical residues in water samples in the rainy and dry season.

Farm	Rainy season			Dry season		
	Paraquat (mg kg^{-1})	Cypermethrin (mg kg^{-1})	Carbendazim (mg kg^{-1})	Paraquat (mg.kg^{-1})	Cypermethrin (mg.kg^{-1})	Carbendazim (mg.kg^{-1})
1	ND	ND	ND	ND	ND	0.090
2	ND	ND	0.010	0.008	ND	0.090
3	ND	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	ND	ND
5	ND	ND	ND	ND	ND	0.009
6	ND	ND	ND	ND	ND	ND
7	0.080	ND	ND	ND	ND	0.060
8	0.010	ND	ND	ND	ND	ND
9	0.006	ND	ND	ND	ND	ND

ND: Lower than detectable limit (concentrations) of 0.005 mg L^{-1} for paraquat and 0.001 mg L^{-1} for cypermethrin and carbendazim

Table 6. Agrochemical residues in chicken blood.

Farm	Paraquat µg L^{-1}	Cypermethrin µg L^{-1}	Carbendazim µg L^{-1}
1	ND	ND	ND
2	ND	ND	ND
3	ND	0.0280	0.1640
4	ND	ND	ND
5	ND	ND	0.0750
6	ND	ND	ND
7	ND	ND	ND
8	ND	ND	ND
9	ND	ND	ND

ND: Lower than detectable limit (concentrations) of 0.001 µg L^{-1} for paraquat, 0.002 µg L^{-1} for cypermethrin and 0.080 µg L^{-1} for carbendazim.

was very less in the soil but most likely found in chicken blood.

Analyses of agrochemical residues in workers' urine samples

Residues in urine were not detectable, not only due to the detectable limit of the instrument but also due to the short stability and long duration of sample transportation and storage (**Table 7**). Besides, urine samples might not be a good specimen for the determination of cypermethrin and carbendazim (*Woollen et al. 1992; Watson 1998*).

Another possibility to explain non-detectable agrochemical residues in human subjects is that the workers always wear long-sleeve shirts, together with facial coverage, glasses, gloves, and boots. These reduced areas to contact with chemicals (*Naksata et al. 2020; Garringou et.al. 2020*). Moreover, the interview revealed that most of the workers were trained related to agrochemical usage and its impacts on health.

Table 7. Agrochemical residues in workers' urine samples.

Farm	Paraquat µg L^{-1}	Cypermethrin µg L^{-1}	Carbendazim µg L^{-1}
1	ND	ND	ND
2	ND	ND	ND
3	ND	ND	ND
4	ND	ND	ND
5	ND	ND	ND
6	ND	ND	ND
7	ND	ND	ND
8	ND	ND	ND
9	ND	ND	ND

ND: Lower than detectable limit (concentrations) of 0.001 µg L^{-1} for paraquat, 0.002 µg L^{-1} for cypermethrin and 0.080 µg L^{-1} for carbendazim.

CONCLUSION AND RECOMMENDATIONS

The study revealed the existence of paraquat, cypermethrin and carbendazim residues in soil and water samples collected in rainy and dry seasons while only cypermethrin and carbendazim were the only residues found in chicken's blood samples. As for the workers' urine samples, concentrations of the three residues were all below the detectable limits of the instruments used and could be also explained by the use of personal protective equipment. In terms of quantification, it was only in soil sample that paraquat and cypermethrin' concentrations were higher than the maximum allowable concentration. Further studies, for examples, a better method for residue analysis and a larger sample size, are needed.

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