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Research Article

Residual and ecological risk assessment of Chlorpyrifos in coffee growing soil areas: A case study in Lam Ha district, Lam Dong province

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Abstract: This study was conducted to investigate the residual of Chlorpyrifos (CPF) in coffee growing soil at Lam Ha district, Lam Dong province. Fifteen soil samples were collected from coffee intensive gardens in the Phi To, Nam Ha, Me Linh and Dong Thanh communes of Lam Ha district during the February 2023. The physico-chemical properties of the samples were analyzed such as pH, organic carbon, moisture content and mechanical composition of the soil while CPF was analyzed by gas chromatography-mass spectrometry. Soil samples with pH ranged from 3.48-4.45, moisture content 28.2-33.8% and OC 2.3-3.4%. Chlorpyrifos concentration of soil samples ranged from not detected to 954 µg/kg. The ecological risk of chlorpyrifos residual in soil was evaluated by using the risk quotient (RQ). The results of this study will be used to propose solutions to reduce pollution.

Keywords: Chlorpyrifos; Pesticides in soil; Soil pollutions; Risk assessment.

1. Introduction

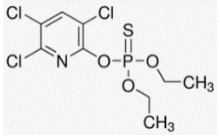
Chlorpyrifos (CPF), also known as Chlorpyrifos Ethyl or O,O-Diethyl O-(3,5,6-trichloropyridin-2-yl) phosphorothioate, is a chemical that belongs to the organophosphorus group is widely used to control pests and diseases. CPF persists for a long time in the environment, has the high bioaccumulation potential, affects the nervous system of humans and other animals [1–3].

CPF is considered as class 2 of hazardous to humans by the World Health Organization. In the United Kingdom, the use of CPF was banned from April 2016. From 2020, CPF was banned throughout the European Union and August 18, 2021, the U.S. Environmental Protection Agency (EPA) announced a ban on the use of CPF on food crops in the United States [4–6]. Due to its high toxicity and adverse effects on the environment, according to Circular 10/2020/TT-BNNPTNT, effective from October 25, 2020: pesticides containing CPF will not be produced or imported, only sold used until February 12, 2021.

In the world, there are many studies on residual as well as risk assessment of CPF in soil, water and in agricultural products. Typical pesticide residue studies include Determination of Chlorpyrifos Residues in Lettuce [7], pesticide contamination in soil of intensive horticulture [8] and pesticide residues in orchard soil [9]. In addition, studies on the

ecological risk of pesticides have been conducted in Ethiopia [10], USA [11], Nepal [12–13] and Iran [14] which have provided important information for future.

Table 1. Physical chemical properties of CPF.

Structure	
Chemical name	O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate
Molecular Weight (g/mol)	350.6
Empirical and Structural Formula	C9H11Cl3NO3PS
CAS Registry Number	2921-88-2
Melting point (°C)	41.5-42.5
Vapor pressure (mmHg)	2.03E-05 at 25°C
Density (g/mL)	1.51 at 21°C
Solubility in water (g/L) at 25°C	1.12
Partition coefficient (n-octanol and water)	log K_{ow} = 5.1

There are very few studies on the residual of pesticides in the soil as well as no research on CPF contamination in the coffee growing soil in Viet Nam. Evaluation of total DDT residues in soil at the plant protection chemical warehouse in Nam Dan, Nghe An [15], residues of pyrethroid pesticides in soil, water, and watercress in Vinh Long [16] and validation of analytical procedures for chemicals such as CPF and carbosulfan in broccoli [17] as well as CPF in soil [18] are among the outstanding studies on the residual of pesticides in Viet Nam.

Lam Ha district is located in the northwest of Lam Dong province, with an altitude of 800-1000 m above sea level, the average annual temperature is about 21-22^{o}C. Lam Ha area is mainly red soil, alluvial soil, and gray soil suitable for industrial crops such as coffee, pepper, vegetables, flowers, and fruit trees. In 2019 and 2020, Lam Ha has a coffee growing area ranking second in Lam Dong province. The plan by 2025 Lam Dong has about 170,000 ha and the output is from 530,000 to 550,000 tons/year with 5 large-scale specialty coffee growing areas: Di Linh, Lam Ha, Bao Lam, Duc Trong, Da Lat city and Lac Duong district.

Lam Ha district is the area with the strongest crop restructuring in Lam Dong province, with a mixture of industrial plants, vegetables, flowers and fruit trees, so the trade in using pesticides is very diverse for many different crops. The transfer of crops also leads farmers to have a habit of using pesticides for short-term plants in combination with coffee plants to prevent pests and diseases. Therefore, the amount and frequency of use of pesticides in Lam Ha will lead to a higher risk of pollution than other coffee farming areas of Lam Dong province.

According to the habit, pesticides are often purchased and stored at warehouses. State agencies only manage at companies and agents that trade in pesticides, but have not fully controlled the situation of using them in warehouses of farmers. Therefore, there are a number of pesticides that have been banned from use, but still in large quantities in households.

The main objectives of the study include: (1) Analysis of physicochemical properties, mechanical composition of soil samples; (2) Analysis of CPF concentration of soil samples; iii) Evaluate ecological risk of CPF.

2. Materials and methods

2.1. Research plan

To achieve these objectives, previous studies on the residues and pollution of CPF were investigated. Soil samples were collected and analyzed for physicochemical properties and

CPF concentration to assess ecological risk. This study also aims to make recommendations to reduce the risk of environmental pollution. The details of the research plan are shown in Figure 1.

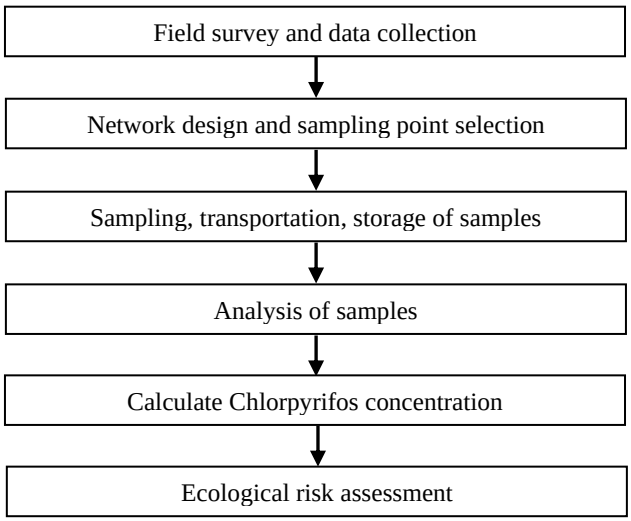


Figure 1. Research diagram.

2.2. Study area

Fifteen soil samples were taken in communes with intensive coffee growing areas of Lam Ha district, namely Dong Thanh, Me Linh, Nam Ha and Phi To at locations as shown in Figure 2. Based on the situation of pests and diseases and the habits of farmers, the sampling time is selected as 3 times per year including February, November (dry season) and June (rainy season).

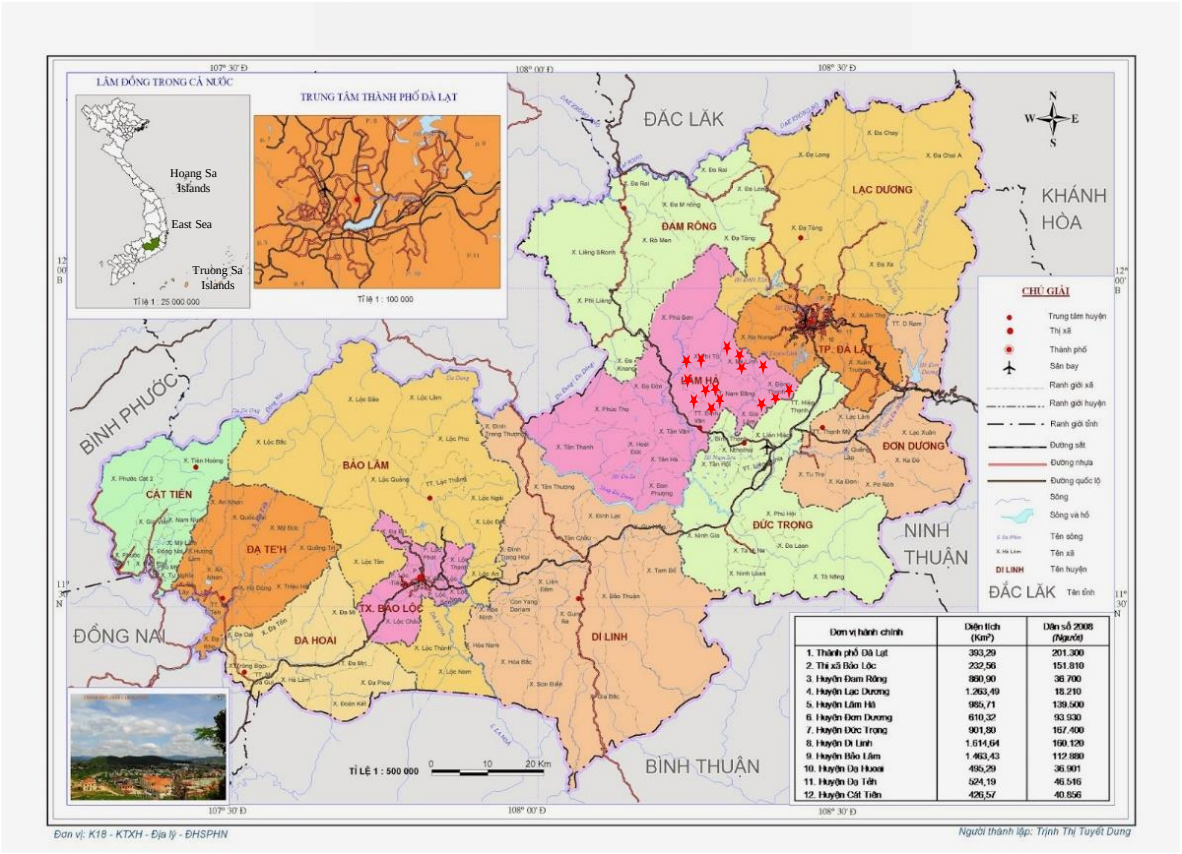


Figure 2. Sampling locations in Lam Ha district.

2.3. Sampling methods

Samples were taken according to the depth from 0-15 cm at the canopy point of the coffee tree to assess the level of retention in the environment of CPF. For square or rectangular gardens, the soil sample is taken diagonally: 1 point is the intersection of 2 diagonals and the remaining 4 points are located on 2 diagonals but about 2-3 rows of coffee trees from the outside. For irregularly shaped gardens, soil samples were taken in a zigzag pattern to ensure representative samples. The number of samples depends on the area of the garden.

At a garden, take 1 bulk sample including at least 5 sub-samples but not more than 10 sub-samples. After removing stones, gravel, roots and leaves, each subsample was taken about 0.5-1 kg, mixed well, and then used the method of quartering to reduce it to about 1kg, put in a dedicated clean nylon bag. Record the sample information and then transfer it to the laboratory for analysis.

2.4. Analysis methods

The pH is determined according to Vietnamese standards TCVN 5979:2021 by soaking the dried, finely crushed samples with a volume of KCl solution at a ratio of 1:5, shaking for about 1 hour and soaking for 1-3 hours, then measure pH at 20±2^{o}C.

Soil moisture is determined according to TCVN 4048:2011 by calculating the percentage of water vapor lost when soil samples are dried at 105^{o}C to constant weight.

Organic Carbon is determined according to TCVN 8941:2011 by oxidation with potassium bichromate solution in concentrated sulfuric acid. Titrate excess potassium dichromate with Fe(II) salt solution. Calculate the result from the weight of the test sample, the concentration of Fe(II) salt solution, the titration volume of the blank and samples.

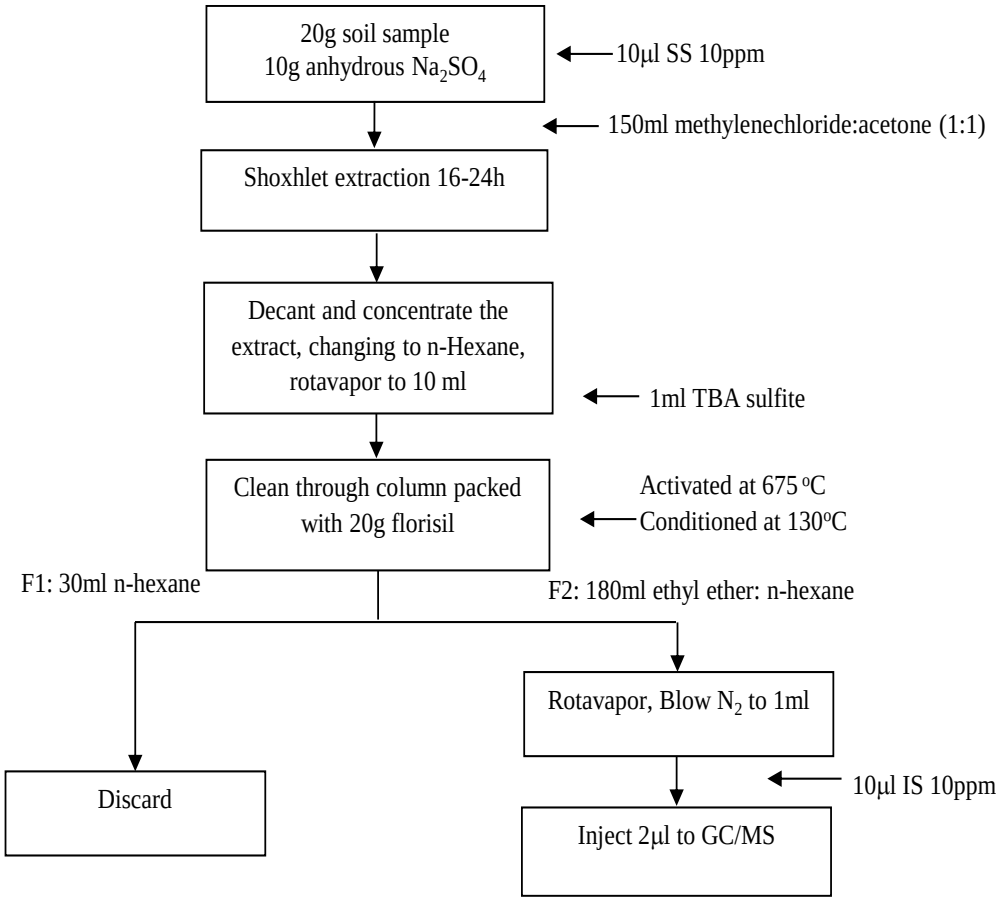


Figure 3. Schematic diagram of analysis of CPF in soil samples.

The mechanical composition of the soil was tested according to Vietnamese standard TCVN 8567:2010. This method uses a mixture of sodium hexamethaphosphate $(NaPO_3)_6$ and sodium carbonate Na_2CO_3 solutions to separate the grain levels of soil samples. Determine the composition of sand by sieve and the composition of limon and clay by pipette. Mechanical composition according to the international system includes 4 levels: Coarse sand (2-0.2 mm), Fine sand (0.2-0.02 mm), Limon (0.02-0.002 mm) and clay (< 0.002 mm).

Analytical methods for concentration of CPF in the soil are: US EPA method 3540C for extraction, US EPA method 3620C for cleanup and US EPA method 8270D for analysis [17–18]. The Schematic diagram for analysis of CPF concentration is depicted in Figure 3. Soil samples were dried, finely crushed and then shoxlet extracted with a mixture of dichloromethane: acetone solvents with a ratio of 1:1 in volume for 16-24 hours. Collect the extract and evaporate to about 10ml and then transfer to n-Hexane solvent. Add TBA sulfite solution to remove S^{2-} in the sample. Clean through a Florisil column containing an anhydrous $NaSO_4$ layer, rinse the column with n-hexane, and then elute with an ethyl ether:n-hexane solvent mixture. Collect the eluate, blow in N_2 stream to final volume 1ml and analyze on GC-MS instrument. The GC-MS operating conditions are shown in Table 2 [19–20]. CPF concentration is calculated from the results of the calibration curve and the soil sample in ug/kg.

Table 2. Operating conditions of GC/MS equipment for the analysis of CPF.

GC conditions	Capillary column: DB5-MS (30m × 0.32mm × 1µm) Program temperature: 70ºC to 150ºC [25ºC/min] → 280ºC [7ºC/min, 4 minutes] Carrier gas: He (99.999%) Sample pump port temperature: 250ºC Sample injection time: 1 minute Column flow rate: 1.7ml/min Sample pump mode: split flow Sample injection volume: 2µl Control mode: linear velocity Line split ratio: 10
MS conditions	Interface temperature: 280ºC Potential for ionization: 70eV Observation mode: Ion selection (SIM)

2.5. Method validation

Repeat the analysis 7-10 times with a sample containing CPF concentration of 3-5 times the estimated limit of detection to calculate the limit of detection (LOD) and the limit of quantification (LOQ). Two analysts performed in parallel to calculate repeatability and reproducibility. Add standards at three levels of concentration: low, medium, and high to calculate the recovery yield. From the results of the repeatability, reproducibility and recovery calculate the uncertainty of the method applied in the laboratory. The method validation results for the determination of CPF in the laboratory were: LOD 1 µg/kg, LOQ 3 µg/kg and uncertainty 11.86%.

2.6. Risk assessment

In this study, the risk quotient (RQ) is used and calculated according to the following formula [13, 23]:

$$RQ = C/ PNEC \quad (1)$$

where C is the concentration of the pollutant analyzed in the sample; PNEC is the concentration value predicted to have no effect on the organism.

$$PNEC = NOEC/ AF \quad (2)$$

where NOEC is the concentration with no effect observed; AF is the rating factor.

Evaluate the levels of risk obtained according to the range of values of the RQ: (1) RQ < 0.01: very low risk; (2) 0.01 ≤ RQ < 0.1: low risk; (3) 0.1 ≤ RQ < 1: moderate risk; (4) RQ ≥ 1: high risk.

3. Results and discussion

3.1. Physicochemical properties of the soil

The soil samples for intensive coffee cultivation in 4 communes of Lam Ha district are acidic, in which Phi To commune has the lowest pH values in the range of 3.48-3.54, Nam Ha and Me Linh communes have similar pH values in the range 3.72-4.45 and 3.96-4.28, respectively. Dong Thanh commune has the highest pH in the range of 4.25-4.33. It is necessary to increase to neutral pH such as lime powder application because the most suitable pH range for coffee growing soil from 4.5-6.0 [24].

The moisture content of 15 soil samples is not much different, ranging from 28.2-33.8%, suitable for plants to grow. The organic carbon concentrations are in the range of 2.3-3.6%. Some samples with low OC% need improvement plans such as in Phi To and Nam Ha communes because the optimal conditions for coffee to grow at an OC% are equal to or greater than 2.5% [24].

All 15 soil samples are mixed clay with clay content of 39.67-66.86. The soil samples in Phi To and Me Linh communes are clay-rich soils with the percentage of mechanical components not much different with the percentages of coarse sand, fine sand, limon and clay in the range of 2.75-2.92, 8.97-11.64, 21.26-22.75 and 62.90-66.86 while in Nam Ha and Dong Thanh communes there is a relatively large difference in percentage of limon and clay. The percentages of coarse sand, fine sand, limon and clay in the soil ranged from 2.49-2.93, 12.58-19.11, 22.83-39.06 and 39.67-61.00, respectively.

3.2. CPF concentration of soil samples

From the weight of the soil sample, the final solvent volume, the mass spectrum in the sample and the standard curve, we can calculate the concentration of CPF with the unit of µg/kg. CPF concentrations of 15 soil samples in 4 communes of Thanh Ha district are presented in Table 3.

Table 3. CPF concentration of soil samples.

Sample location	Number of Sample	CPF concentration (µg/kg)		
		Min	Average	Max
Phi To communes	3	Not detected	31.7	65.7
Nam Ha communes	5	Not detected	75.5	175
Me Linh communes	4	389	543	954
Dong Thanh communes	3	422	593	821

From CPF concentration of fifteen soil samples in Lam Ha district, we found that Phi To and Nam Ha communes have lower CPF levels than the other two communes, Me Linh and Dong Thanh. Soil samples in Phi To commune had the lowest CPF concentration with 1 sample not detected and maximum concentration was 65.7 µg/kg. Nam Ha Commune had 2 samples without CPF detected, the average concentration is twice as high as that of Phi To commune and the maximum concentration is 175 µg/kg. The CPF concentrations of soil samples in Me Linh and Dong Thanh communes ranged from 389 to 954 µg/kg, higher than previous research results.

Although CPF has been banned in Vietnam since February 2021, some pesticide suppliers and farmers continue to trade and use it illegally. The main source of CPF in soil

samples can be from pesticides used to control pests and diseases at the beginning of the rainy season every year or can be remained from sprays before. Therefore, it is necessary to continue monitoring to evaluate the residual of CPF comprehensively.

CPF concentration is compared with result of previous studies in Table 4. The results obtained from this study were uneven: Some samples did not detect CPF but some samples were very high. These were substantially higher than a study of rice grow land at the Mekong Delta and studies at Nepal and Iran in 2021. This CPF result was lower than studies at Malaysia in 2010.

Table 4. CPF concentration (µg/kg).

Sample Locations	Type of sample	Year of sampling	CPF concentration (µg/kg)	Reference
Lam Ha	Soil	2023	Not detected - 954	This study
Mekong Delta	Land	2019	3.51-291	PhD thesis summary
Nepal	Soil	2021	32.5 - 177	Bhandari, G. [13]
Iran	Soil	2021	240-510	Arani, M.H. [14]
Malaysia	Land	2010	20 - 2240	Tahir, N.M. [25]

3.3. Risk assessment

With AF equal to 10 and NOEC equal to 65 (µg/kg) [23], risk index RQ were calculated in Table 5.

Table 5. Risk assessment.

Sample name	CPF concentration (µg/kg)	RQ	Risk assessment
Phito communes	Not detected-65.7	<1.0-10.1	Moderate- high risk
Nam Ha communes	Not detected-175	<1.0-26.9	Moderate- high risk
Me Linh communes	389-954	59.8-146.8	High risk
Dong Thanh communes	422-821	64.9-126.3	High risk

The results of the RQ index show that the ecological risk impact of CPF contamination in coffee soils in Lam Ha district is moderate to high and requires mitigation measures. The order of the communes with the risk from moderate to high in this study is Phi To, Nam Ha, Dong Thanh, and Me Linh, respectively. Soil samples detected with CPF are all moderate-highly toxic. Ecotoxicological information on pesticides and degradation products is not always available and should be incorporated in risk assessments. Furthermore, the ecotoxicity of the degradation product of CPF is higher than that of CPF itself. Higher level risk assessment methods to improve assessment risk should also be considered for a better understanding of pesticides.

4. Conclusion

The physicochemical parameters, mechanical composition and CPF concentration in 15 samples of coffee growing soil in Lam Ha were archived. CPF residual ranged from not detected to 954 µg/kg. This study assesses the ecological risk of CPF in soil based on the RQ risk quotient method, result showed all moderate-highly toxic. Due to the limited number of studies on risk assessment in Vietnam on CPF and other pesticides, it is necessary to promote studies in this direction using assessment methods such as: RQ risk quotient method, CR

cancer risk index method, distribution modeling method, matrix method to provide more information for authorities to manage the use of pesticides in agriculture and forestry. Further studies need to be carried out on a large scale with an increased number of samples and type of pesticides to get a comprehensive view of the status of pollution and ecological risks of pesticides in coffee growing soil in Lam Ha district.

Authors contribution: Constructing research idea: D.V.L., V.D.T.; Select research methods: D.V.L., V.D.T.; Data collection: D.V.L., V.D.T.; Data processing: D.V.L., V.D.T.; Writing original draft preparation: D.V.L.; Writing review and editing: D.V.L., V.D.T., T.M.P.

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Conflicts of interest: The authors declare that this article was the work of the authors, has not been published elsewhere, has not been copied from previous research; there was no conflict of interest within the author group.

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