



## Organochlorine Pesticides Residue Level in Surface Water of Cameron Highlands, Malaysia

M. P. Abdullah<sup>1,2\*</sup>, Y. F. Abdul Aziz<sup>1,2</sup>, M. R. Othman<sup>1,2</sup>, W. M. A. Wan Mohd Khalik<sup>1</sup>

<sup>1</sup>School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Malaysia

<sup>2</sup>Centre for Water Research and Analysis (ALIR), Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Malaysia

### PAPER INFO

#### Paper history:

Received 22 January 2015

Accepted in revised form 1 March 2015

#### Keywords:

Endocrine disruptor chemicals

Organochlorine pesticide

Persistent organic pollutant

Surface water

### ABSTRACT

The occurrence of organochlorine pesticide (OCPs) residues in surface water of Bertam and Terla River, Cameron Highlands was investigated. Monitoring work was carried out starting from August to December 2014 consisted of six stations. Nine of organochlorine residues were detected throughout that period of study, namely aldrin, heptachlor epoxide, endosulfan II, endosulfan sulfate, methoxychlor, endrin ketone, 4,4'-DDE, beta and gamma HCH. The level concentration of pollutant were found to be in the order endrin ketone (mean  $0.317 \mu\text{gL}^{-1}$ ) > beta HCH ( $0.153 \mu\text{gL}^{-1}$ ) > endosulfan sulfate ( $0.112 \mu\text{gL}^{-1}$ ) > endosulfan II ( $0.072 \mu\text{gL}^{-1}$ ) > aldrin ( $0.071 \mu\text{gL}^{-1}$ ) > methoxychlor ( $0.06 \mu\text{gL}^{-1}$ ) > heptachlor epoxide ( $0.023 \mu\text{gL}^{-1}$ ) > 4,4'-DDE ( $0.011 \mu\text{gL}^{-1}$ ) > gamma HCH ( $<0.002 \mu\text{gL}^{-1}$ ). The highest total organochlorine residue is detected at  $0.955 \mu\text{gL}^{-1}$ . The highest concentrations for compounds namely methoxychlor ( $0.06 \mu\text{gL}^{-1}$ ), endrin ketone ( $0.63 \mu\text{gL}^{-1}$ ) and 4,4'-DDE ( $0.018 \mu\text{gL}^{-1}$ ) were found at station S2 (Kuala Terla). An analysis in tap water (station S5; Brinchang town) also found some compounds namely aldrin, endosulfan II, endrin ketone and 4,4'-DDE to be within the range of 0.008 to  $0.18 \mu\text{gL}^{-1}$ . To mitigate this present status from continuing to become worse, improving water quality for water supply or reuse for agriculture purpose in Cameron Highlands must be put in high priority among all stakeholders.

doi: 10.5829/idosi.ijee.2015.06.02.10

### INTRODUCTION

Pesticides are categorized into many classes such as organochlorine, organophosphorus, carbamate, pyrethroid and etc. One of the most common traceable pesticides in our environment are organochlorine pesticides (OCPs) particularly which had known to be a persistent organic pollutant (POPs). In 2001, initially 12 POPs had been listed by UNEP, 9 of which were OCPs. Later, a new revision in Stockholm Convention 2009 listed additional compounds up to 15 OCPs group [1, 2]. Out of 40 types of banned pesticides listed by the Malaysian Ministry of Agriculture, some of them are chlorinated pesticides namely alachlor, aldrin,

chlordane, DDT, dieldrin, endosulfan, HCH isomers and heptachlor<sup>1</sup>.

Pesticide residues such as organochlorines can exist in our aquatic environment through precipitation, runoff and leaching from nearby agriculture field. Mobility of this residue can be influenced by their chemical characteristics such as low water solubility, low biodegradation rate, and strong affinity for particulate matter [3]. Detrimental effect on non-targeted organisms, degradation of environmental quality and threat as endocrine disruptors are few ubiquitous examples due to this type of pesticides [4].

Despite the prohibited usage of this type of pesticide in many countries including Malaysia, obedience to the rule and regulations is still doubtful. Economic survivor factors such as low price, effective and easy access are the contiguous factors being considered by farmers.

\* Corresponding author: Md Pauzi Abdullah  
E-mail: mpauzi@ukm.edu.my

<sup>1</sup> <http://www.doa.gov.my/maklumat-racun-perosak> [2 July 2014]

Several monitoring projects have been performed in the Asian countries especially to investigate the ubiquitous impact of banned pesticides residues on water and sediment quality of rivers namely Han River, South Korea [5], Gomti River, India [6], Daliao River and Qiantang River, China [7, 8], as well as in the ASEAN countries such as in Red and Duong Rivers (Vietnam), Mae Klong River and Patani River, Thailand [9-11].

As part of the global countries, Malaysia has also experienced similar problems. One of the most developed areas for extensive agricultural activities is known as Cameron Highlands. This highlands area is located at 4°31"N and 101°29" E, which is situated in Pahang, east coast of Peninsular Malaysia. Major rivers flow across this area namely Bertam, Burong, Telom, Lemoi, Habu, Tringkap and Terla River. Deterioration of water quality in this area has been reported widely. In relation to agricultural area, occurrences of banned pesticides are a great concern. Therefore, the objective of present work is to evaluate the organochlorine residues level especially the occurrences of banned pesticides in water resources of Cameron Highlands in relation to human consumptions.

## MATERIAL AND METHODS

### Chemical and Reagent

Seventeen individual reference analytical grade standards namely 4,4'-DDT and metabolites (4,4'-DDD, 4,4'-DDE) HCH isomers (alpha, beta, and gamma), endosulfan (I, II and sulfate), heptachlor, heptachlor epoxide, methoxychlor, endrin, endrin ketone, endrin aldehyde, aldrin and dieldrin were obtained from Supelco (Bellefonte, USA). All solvents used for sample extraction and analysis (methanol, ethylacetate, dichloromethane) were pesticide grade (Merck). Resprep C18 6 mL 500 mg solid phase extraction tube (Restek, USA) was chosen for sample extraction throughout the period of study. Deionized water was obtained by using a Milli-Q Easypure Rodi system (Barnstead, USA).

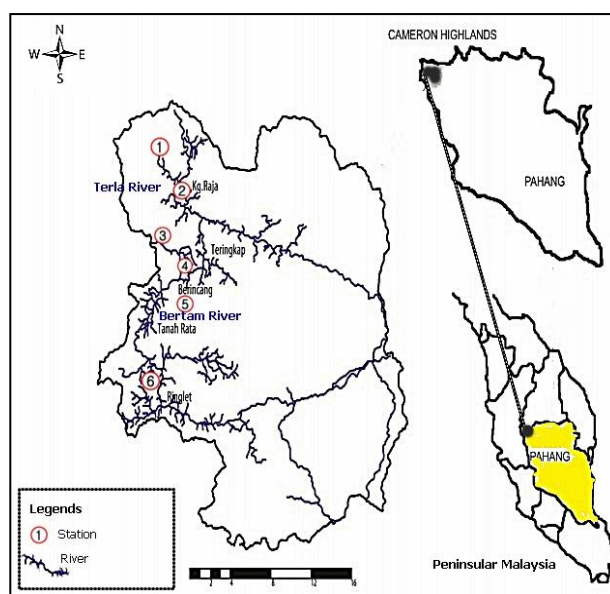
### Sample Collection and Analysis

Surface water samples were taken monthly from Terla and Bertam Rivers in Cameron Highlands starting from August to December 2014. Six stations were chosen and descriptive locations for each site are presented in Figure 1 and Table 1. Water samples were collected using a 1 L glass bottle. It was kept in a cool box prior to being transferred to laboratory for further analysis. Onsite measurement was also taken using YSI 556 multiprobe sensors (YSI, USA) for parameters namely pH, temperature, dissolved oxygen, conductivity, salinity and dissolved solids.

Water samples were extracted based on our established and validated method from previous work [12]. In general, samples were filtered using 0.45  $\mu\text{m}$  GF/C membrane filters to remove suspended particulate. SPE tube cartridge (Resprep C18) was initially conditioned with 10 mL methanol, 5 mL ethylacetate, 5 mL dichloromethane and 10 mL deionized water. One litre of the sample was then loaded continuously into the tube with the flow rate of 1.5 mL  $\text{min}^{-1}$ . The cartridge was then eluted with 5 mL ethylacetate and 5 mL dichloromethane. The eluted sample was placed under nitrogen stream until completely dryness. Concentrated sample was obtained after reconstituted with 1 mL n-hexane. Sample was kept in amber vials before it was ready to be injected into the gas chromatography. A series of stepwise dilution solutions (range 6.5 – 102  $\mu\text{gL}^{-1}$ , n=7) were used for the construction of external calibration curves in order to determine unknown concentrations of targeted compounds. Other analytical figures of merit, such as the limit of detection and quantification, were also performed.

**TABLE 1.** Descriptive of sampling location in Cameron Highlands

Station Code	Area	Latitude	Longitude
S1	Kuala Terla	04° 33.466 N	101°24.258 E
S2	Kuala Terla	04° 32.543 N	101°25.989 E
S3	Taman Sedia	04° 28.771 N	101°23.244 E
S4	Parit Fall, Brincang	04° 28.442 N	101°23.423 E
S5	Brincang Town	04° 29.283 N	101°23.175 E
S6	Habu	04° 21.334 N	101°22.676 E



**Figure 1.** Map of Cameron Highlands indicates sampling stations

Varian CP3800 Gas Chromatography – Electron Captured Detector (GC-ECD) equipped with HP-5ms capillary column (30 m x 250  $\mu\text{m}$  x 0.25  $\mu\text{m}$  thickness) was used for chromatographic separation of the targeted pesticide. Detail description of the instrument setting is summarized in Table 2.

**TABLE 2.** Instrument setting for analysis of targeted compounds

Aspect	Setting
Injection Volume	1 $\mu\text{L}$
Injection Temperature	250°C (splitless mode)
Detector Temperature	300°C
Oven Program	Initial 90°C to 170°C (ramped with 3.5°C $\text{min}^{-1}$ ), then up to 280°C (ramped with 5°C $\text{min}^{-1}$ ). Total runtime is 45.86
Flow rate	1.5 $\text{mL min}^{-1}$ (99% purified nitrogen)
Make up flow	25 $\text{mL}$

## RESULTS AND DISCUSSION

### In-situ Measurement

The variations of physical water chemistry for Bertam and Terla River are shown in Table 3. The mean concentrations of on-site measurement showed small variation as compared to the previously reported work. Parameters namely temperature, dissolved oxygen and salinity had slightly increased throughout the period of study. This present status indicated that water chemistry has not experienced drastic deterioration of water quality over the past years.

**TABLE 3.** Descriptive of in-situ measurement for surface water

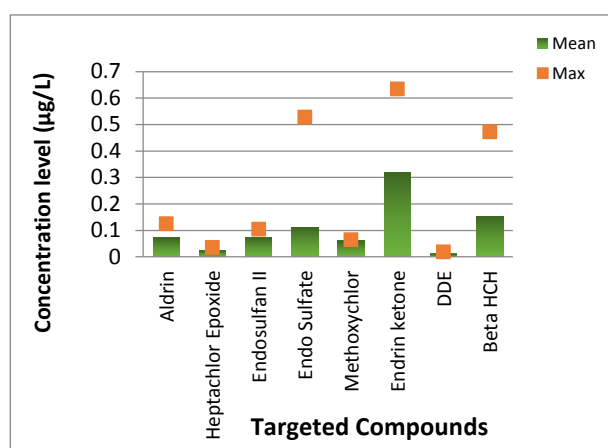
Parameter	Range	Mean (SD)	Previous Study <sup>a</sup>
pH	5.43 – 6.92	6.35 (0.57)	6.54 (0.15)
Temperature (°C)	18.45 – 21.04	19.67 (0.99)	18.18 (0.91)
Conductivity ( $\mu\text{Scm}^{-1}$ )	29.25 – 64.2	54.98 (14.60)	58.33 (9.19)
Dissolved Oxygen ( $\text{mgL}^{-1}$ )	7.46 – 9.23	8.48 (0.68)	6.31 (1.87)
Dissolved Solids ( $\text{mgL}^{-1}$ )	18.25 – 43.01	35.86 (1.01)	43.33 (7.31)
Salinity (ppt)	0.01 – 0.03	0.02 (0.007)	0.01(0.002)

<sup>a</sup> Khalik et al [13]

### Level of Concentration of Pollutants

Analysis of water samples showed only 9 compounds detected throughout the period of study namely aldrin, heptachlor epoxide, endosulfan II, endosulfan sulfate, methoxychlor, endrin ketone, 4,4'-DDE, beta and gamma HCH. Sum of peak for all compounds could be grouped into six chemical structures based on classes *Hexachlorooctahydronaphthalene* (aldrin, endrin ketone), *Chlorinated camphene* (heptachlor epoxide),

*Chlorinated diphenylethane* (methoxychlor), *Hexachlorocyclohexane* (beta and gamma HCH), *chlorinated cyclodiene* (endosulfan II, endosulfan sulfate) and *DDT isomers* (4,4'-DDE). The highest total organochlorine residue detected was 0.955  $\mu\text{gL}^{-1}$ . Level concentration of pollutant was found to be in the order endrin ketone > beta HCH > endosulfan sulfate > endosulfan II > aldrin > methoxychlor > heptachlor epoxide > 4,4'-DDE > gamma HCH. Mean concentration of each compound is illustrated in Figure 2.

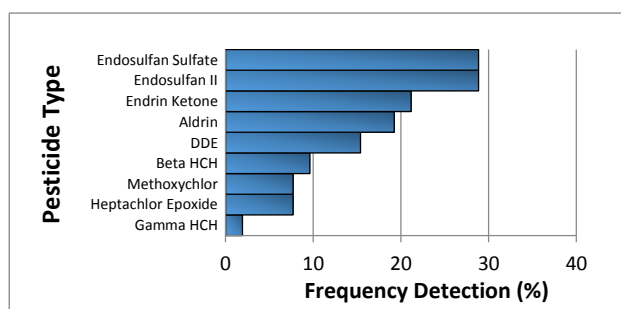


**Figure 2.** Mean and maximum concentrations detected for each compound

The highest detected concentration of organochlorine residue in water was endrin ketone. It ranges from not detected (ND) to 0.634  $\mu\text{gL}^{-1}$  (mean 0.317  $\mu\text{gL}^{-1}$ ) with the frequency of detection of 21.15%. Despite of remarkable detection for metabolite endrin ketone in water samples, their primary substance endrin was not abundant in this study. Among HCH isomers, only beta and gamma HCH were observed in water samples. For beta HCH, it ranged from ND to 0.472  $\mu\text{gL}^{-1}$  (mean 0.153  $\mu\text{gL}^{-1}$ ). Frequency of detection of this compound was 9.61%. This type of pesticide is relatively resistant to microbial degradation. Moreover, its chemical properties have the lowest solubility and vapour pressure compared to other isomers. Therefore, beta HCH is more stable in aquatic environments [10]. It has been stated that alpha HCH can also be converted to beta HCH in certain conditions [14]. However, one time detectable of gamma HCH was below than the limit of quantification (LOQ < 0.002  $\mu\text{gL}^{-1}$ ).

In the case of endosulfan group, only secondary component (endosulfan II) and degradation by product (endosulfan sulfate) were detected in all sampling points with the frequency of detection was 28.84% for both compounds. The level of concentration ranged from ND to 0.104  $\mu\text{gL}^{-1}$  (mean 0.072  $\mu\text{gL}^{-1}$ ) for endosulfan II and from ND to 0.527  $\mu\text{gL}^{-1}$  (mean 0.112  $\mu\text{gL}^{-1}$ ) for

endosulfan sulfate. The hydrolysis of endosulfan was known to be pH dependent which is increased by a factor of 10 for each increase in pH value above 7. Endosulfan sulphate, however, broke down much slow and it was not as strongly influenced by pH level as endosulfan [15]. Thus, there was no surprise that the level of concentration for endosulfan sulfate was higher than the primary substance. It can be related to present of water chemistry has shown mean pH level below than 7. Aldrin residues were detected in level ranging from ND to  $0.124 \mu\text{gL}^{-1}$  (mean  $0.071 \mu\text{gL}^{-1}$ ). The level of mean concentration for aldrin residues in water samples exceeded the limit set by WHO ( $0.03 \mu\text{gL}^{-1}$ ). Frequency of detection of this compound was 19.23%, as presented in Figure 3. Although aldrin was detected in many samples, isomeric ratio could not be accounted because dieldrin was not detected throughout the period of study. Methoxychlor and heptachlor epoxide had been found at a low level concentration with the frequency of detection was only at 7.69 %. For methoxychlor compounds, it ranged between ND to  $0.064 \mu\text{gL}^{-1}$  (mean  $0.060 \mu\text{gL}^{-1}$ ). Level of concentration obtained for heptachlor epoxide was ranged between ND to  $0.035 \mu\text{gL}^{-1}$  (mean  $0.023 \mu\text{gL}^{-1}$ ). No detectable amount of primary substance heptachlor was found in all water samples.



**Figure 3.** Frequency of detection (%) for each targeted compounds

Both 4,4'-DDD and 4,4'-DDE are known to be the degradation products of 4,4'-DDT. It is not surprising to detect 4,4'-DDE, which is less degradable compound compared to other metabolites. In case of 4,4'-DDT group, only 4,4'-DDE was observed with the level of concentration from ND to  $0.018 \mu\text{gL}^{-1}$  (mean  $0.011 \mu\text{gL}^{-1}$ ).

### Temporal and Spatial Variation

Temporal and spatial variation of OCPs residues were investigated in this study. The total number of pesticides detected in the samples was the highest at station S1 and S2, which accounted for 6 compounds namely aldrin, heptachlor epoxide, endosulfan II, endosulfan sulfate, methoxychlor and endrin ketone. The highest

concentrations for compounds namely methoxychlor ( $0.06 \mu\text{gL}^{-1}$ ), endrin ketone ( $0.63 \mu\text{gL}^{-1}$ ) and 4,4'-DDE ( $0.018 \mu\text{gL}^{-1}$ ) were found at station S2. Both stations were situated at Terla River, facing flow across an extensive agricultural activity. The standard of chromatogram peak of the water sample at S1 is illustrated in Figure 4.

Other sampling stations (S3, S4) also showed similar spatial pattern with less contribution of pollutant. Although this area has also experienced land use for vegetables production, it was not as extensive as in the upper region area, the Blue Valley. Furthermore, this area was more developed for human settlement, known as the Brincang town. In downstream of the Bertam River, station S6 also presented some compounds such as endosulfan sulfate, endrin ketone and 4,4'-DDE with varying level of concentrations.

An analysis of samples collected from station S5 is a great area of concern. Sample from this station was remarked as tap water, directly collected from the pipelines, which mean that it was also being used as drinking water for the local populations. In this study, some compounds namely aldrin, endosulfan II, endrin ketone and 4,4'-DDE were detected at least once in the water samples. It must be stressed that, although the concentration of pesticides in pollutant was very low ( $\mu\text{gL}^{-1}$ ), it can still cause harmful effects. Therefore, improving the quality of water in Cameron Highlands for water supply or reuse for agriculture purpose must be put as a high priority. The temporal and spatial distribution of  $\Sigma\text{OCPs}$  in surface water is illustrated in Figure 5. In general, the frequency of detection for total OCPs was higher during August to October rather than during the sampling period in November. Distribution of  $\Sigma\text{OCPs}$  was less in the month of November because the sample was collected during rainy days. This would give flux dilution effect, which loaded relatively low levels of  $\Sigma\text{OCPs}$ . In December, beta HCH was detected in all water samples except at S2. We may presume that this compound was reintroduced by the suspension of sediments since no primary substance 4,4'-DDT was found during the period of study. However, analysis of sediment samples was not conducted, thus, supplementary data was not obtained to support this assumption.

Analysis of Variance (One way ANOVA) indicates that spatial variation had no significant difference during the period of study. However, for the temporal variation, the collected data had shown significant differences with ( $p < 0.05$ ,  $p = 0.008$ ) at 95% confidence level. This outcome means that meteorological input, such as precipitation, has influenced not only on the occurrence of pesticide compounds in surface waters but on the concentrations level of pollutants as well.

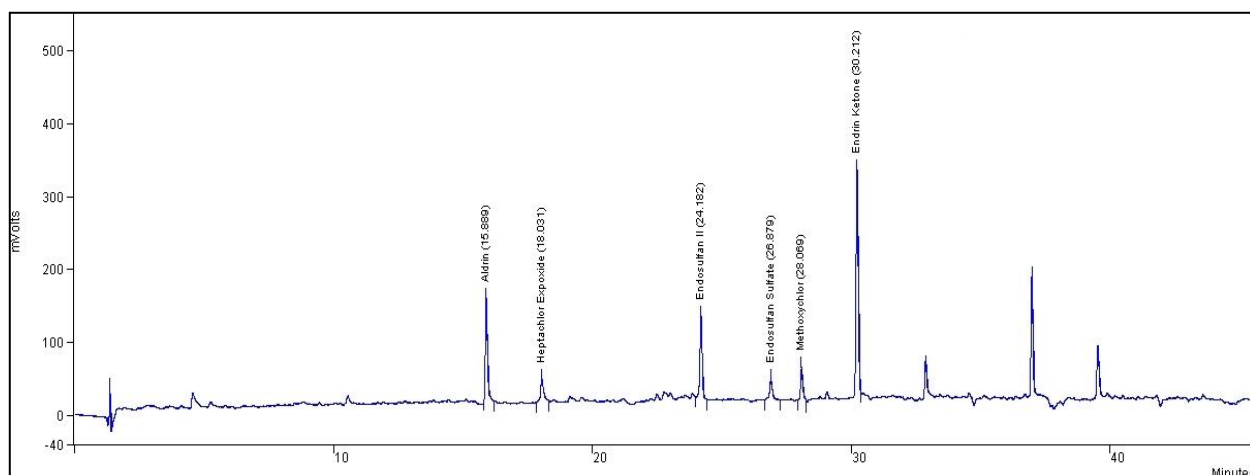


Figure 4. Chromatogram peak of pesticide detected from station S1

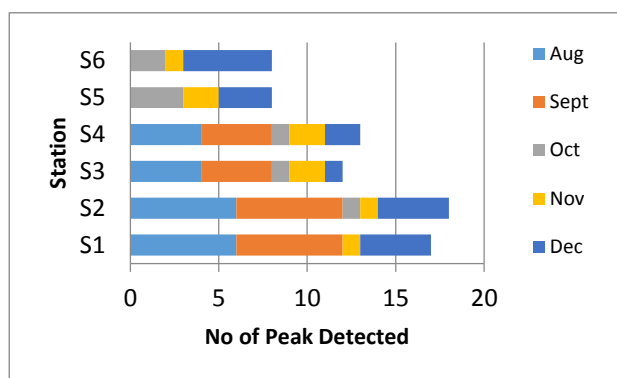


Figure 5: The temporal and spatial variation of pesticide detected

### Comparison with other studies

Comparison with other previous reports done in this study area is summarized in Table 4. In general, the levels of concentration found in this study are quite low compared to the pesticide levels found in the river of Iran and Thailand. All of the study areas experienced extensive agriculture activities nearby the river basin. In most of the cases of past studies, endosulfan sulfate was not detected but it is present remarkably in this study. Although the low level of concentrations for Tonghui River was presented here, this compound was not dominant in their study. According to Zhang et al. [16], the Tonghui River was polluted with the highly dominant by HCH isomers, benzene hexachloride isomers and heptachlor. Total concentration of OCPs was  $704 \text{ ngL}^{-1}$ . Neighbourhood country, Thailand also have similar experienced, which detected higher concentrations than present study especially 4,4'-DDE pollutant in Patani River.

TABLE 4. Level of pollutants in surface water from different rivers in Asian

Compounds	Level of Concentration ( $\mu\text{gL}^{-1}$ )			
	This study (Malaysia)	Patani River (Thailand) <sup>a</sup>	Tonghui River, (China) <sup>b</sup>	Karun River (Iran) <sup>c</sup>
Aldrin	0.071	1.85	0.018	0.79
Beta HCH	0.153	3.72	0.056	1.81
4,4'-DDE	0.011	11.29	0.025	NA
Endosulfan II	0.072	NA	0.067	28.5
Endosulfan Sulfate	0.112	NA	NA	NA
Endrin Ketone	0.317	0.14	0.004	NA
Heptachlor	0.023	1.70	0.004	NA
Epoxide				
Methoxychlor	0.060	NA	0.005	5.44

NA – Not Available; <sup>a</sup> Samoh and Ibrahim [11]; <sup>b</sup> Zhang et al [16]; <sup>c</sup> Behfar et al [17]

### CONCLUSION

An assessment of surface water in Cameron Highlands was successfully carried out. Nine out of 17 targeted compounds were detected throughout the period of study. Despite the occurrence of the targeted compounds at low concentrations levels, more detailed work has to be carried out in the future. Since water supply is human necessity in daily life, any contaminant should be reduced or avoided. In order to ascertain more information about water supply, our work needs to be extended to treatment plants in Cameron Highlands, which are located in Habu and Brinchang. Future work in Cameron Highlands can also be extended to other types of pesticides namely carbamate, organophosphorus or pyrethroid, which will also give ubiquitous negative impacts on human and environmental ecosystems.



## ACKNOWLEDGEMENT

Authors are thankful to the Pesticide Action Network for Asian and The Pacific (PANAP) and Japan Fund for Global Environment (JFGE) for financial research support. Gratitude is also extended to PANAP members, which assisted our team during the fieldwork.

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## Persian Abstract

DOI: 10.5829/idosi.ijee.2015.06.02.10

## چکیده

وقوع باقیمانده حشره کش های ارگانوکلر در سطح آب رودخانه Betram و Terla، Cameron Highlands مورد تحقیق قرار گرفت. نظارت کار در شش مکان از آگوست آغاز شد و تا دسامبر ۲۰۱۴ ادامه داشت. نه باقیمانده از ارگانوکلر مانند آلدین، هپتاکلر اپوکسید، اندوسولفان ۲، سولفات اندوسولفان، متوکسی کلر، کتون اندرین، HCH، 4,4'-DDE بتا و گاما در زمان مطالعه تشخیص داده شد. سطح غلظت آلوده کننده ها باید برابر مقدارهای معین باشد، کتون اندرین (متوسط  $0.317 \mu\text{gL}^{-1}$ ) < بتا HCH ( $0.153 \mu\text{gL}^{-1}$ ) < سولفات اندوسولفان ( $0.112 \mu\text{gL}^{-1}$ ) < اندوسولفان ۲ ( $0.072 \mu\text{gL}^{-1}$ ) < آلدین ( $0.071 \mu\text{gL}^{-1}$ ) < متوکسی کلر ( $0.06 \mu\text{gL}^{-1}$ ) < هپتاکلر اپوکسید ( $0.023 \mu\text{gL}^{-1}$ ) < 4,4'-DDE ( $0.011 \mu\text{gL}^{-1}$ ) < گاما HCH ( $0.002 \mu\text{gL}^{-1}$ ). بالاترین میزان کشف شده از کل ارگانوکلر باقیمانده  $0.955 \mu\text{gL}^{-1}$  بود. بالاترین میزان غلظت ترکیباتی مانند متوکسی کلر ( $0.06 \mu\text{gL}^{-1}$ )، کتون اندرین ( $0.63 \mu\text{gL}^{-1}$ ) و 4,4'-DDE ( $0.018 \mu\text{gL}^{-1}$ ) در مکان S2 (Kuala Terla) یافت شد. از آنالیز آب بهره برداری شده (مکان S5: Brinchang town) همچنین ترکیباتی مانند آلدین، اندوسولفان ۲، کتون اندرین و 4,4'-DDE در محدوده ۰/۰۰۸ تا  $0.18 \mu\text{gL}^{-1}$  یافت شد. برای سبک کردن وضعیت موجود از ادامه یافتن و بدتر شدن آن، بهبود کیفیت آب برای تامین آب یا استفاده مجدد آن به منظور کشاورزی در Cameron highlands باید در اولویت بالا نسبت به عوامل دیگر قرار گیرد.