Analysis of Pesticide Residues in Water Sample: Occurrence of Pesticides in Paddy Field

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ABSTRACT

Pesticides have been used extensively by the farmers in Malaysia during the last few decades.As Organochlorine (OCPs) and organophosphorus (OPPs) pesticides have been used commonly in the agriculture field, a straightforward and efficient method of extraction for pesticides using solid phase extraction (SPE) have been developedfordetection of OCPs and OPPs in water. Seventeen selected compounds of OCPs and OPPs were extracted from water using RP-C18 cartridge and the conditions of extraction were optimized. All the pesticides were analyzed using gas chromatography-electron captured detector (GC-ECD). The sample recoveries were excellent for most pesticides with a range of 74.2 to 116.4 % at two levels concentrations of 20 and 50µg/L. The optimum conditions for extraction were obtained using of 1000 mL of sample volume, 10 mL of 1:1 (methanol: free organic water) for conditioning and 10 mL of 1:1 (n-hexane: acetone) for elution at 3 mL/min of flow rate. The limit of detection LODs range from 0.01to 0.088µg/L and the limit of quantification LOQs range from 0.035 to 0.290µg/L. RSD% ranged from 1.25 to 6.80. Calibration curve was built at concentration ranged from 50 to 500µg/L with good correlation coefficients (R^2) of0.992 to 0.998.Forty water samples, collected from both organic and conventional paddy fields in Ledang, Johor, Malaysia were analyzed to determine the occurrence and distribution of OCPs and OPPs pesticide residues. Pesticide residues detected in conventional field vary from 0.081 to 0.695µg /L which is slightly higher than those detected in organic fields from (0.058 to 0.662) µg/L. This is due mainly from the historically used of pesticides in the plot although no pesticide were used in the organic plot since the last three years. This clearly indicates that the pesticide residues in the soil from historical used can still contaminate the organic field environmental.

KEY WORDS: Pesticide, Environmental Analysis, Gas Chromatography, SPE.

1. INTRODUCTION

Pesticides are chemicals used in modern agricultureto control, kill pest species and to increase crop production as part of pest control strategy, which promotes the public health by combating certain human diseases transmitted by insect vectors (Margni, 2002). The introduction of pesticides in agriculture has helped to increase productivity and has thus contributed to steadily rising food production since the Second World War. Fungicides and insecticides applications have led to an increase in the yields in arable farming, and also avoid losses during storage of the products at the same time (Moscat and Diaz-meco, 2009).

Organochlorine pesticides (OCPs) such dichloro-diphenyl-trichloroethane (DDT) are very stable substances and very persistent. The degradation in soil is about 75-100% in the space of 4-30 years. Due to its long persistence in the environment, their toxicity and hazards, there is a considerable interest in examining the pollution of OCPs in environment (Golfinopoulos, 2003). Globally, several hundreds of pesticides are currently used for agricultural purposes, and they are detected by determination of their residues in various environmental matrices, such as soil, water, and air. Pesticides are divided into many classes, of which the most important are Organochlorine and organophosphorus (OPPs) compounds (Zulin, 2002). In contrast to OCPs; OPPs degrade rapidly depending on their chemical formulation, method of application, climate and the growing stage of the plant. Pesticide residues reach the aquatic environment through erosion as a direct run-off, leaching, improper disposal of used containers and utensils (Miliadis, 1994).

Pesticides can also be moved from agricultural fields to surface waters in surface run-off (Tolosa, 1996). The amount lost from fields and transported to surface waters depends on several factors including soil property, topography, weather, and agricultural practices, chemical and environmental properties of individual pesticides (Konstantinou, 2006).

Various methods have been developedfor the extraction of OCPs and OPPs in water such as liquid-liquid extraction (LLE) (Kishimba, 2004), solid-phase extraction (SPE), solid-phase microextraction (SPME), stir bar sorption extraction (SBSE) and liquid-phase microextraction (LPME). The need for strict experimental control and long equilibrium times, however, limits the application of SPME, SBSE, and LPME in large-scale analysis of field-collected water samples. Besides, SPME is quite expensive and the polymer coating in SPME is fragile and easily broken which need more precaution in handling it. For LLE, it is time-consuming, labor-intensive and can introduce errors and losses when analyzing volatile compounds (Karoly and Gyorgy, 2004).

The SPE extraction cartridge is especially appealing for use in pesticide residue analytical methods since it was reported to achieve good recoveries for most of the pesticides (Stajnbaher and Zupan, 2003). SPE cartridges are usually utilized for extraction of pesticides from a water matrix or for purification of pesticide extract. With the right SPE cartridge, the pesticides of interest are adsorbed on the sorbent bed while the interferences pass through without retention. The pesticides are then eluted and carried forward to an appropriate determination step. Reversed phase involves a polar or moderately polar sample matrix (mobile phase) and a nonpolar stationary phase. The analyte of interest is regularly mid- to nonpolar. Retention of organicanalytes from polar solutions (e.g. water) onto these SPE materials is due to the attractive forces between the carbon-hydrogen bonds in the analyte and the functional groups on the sorbent surface. These nonpolar − nonpolar attractive forces are commonly called van der Waals forces or dispersion forces (Biziuk, 2006).

In developing a method for pesticides extraction by SPE, several factors need to be considered in effecting recoveries of the pesticides. There are four steps in solid phase extraction which are prewash or column preparation, sample loading (retention), column post-wash and finally analyte desorption (elution). Recovery is calculated by comparing the original concentration to the concentration after solid phase extraction. It is a function of both retention efficiency and elution efficiency (Bhuiyan and Brotherton, 2002).

For instrumental analysis, gas chromatography (GC-ECD) is the most popularly utilized procedures for the identification andquantification of pesticides in water. However, inspite of the recent technical progress and due to very low levels of pesticide residues in water samples, the pesticide quantification in water requires a first step of extraction and preconcert ration. This sample preparation step is, in fact, the critical step of the whole analysis. The most common method is solid- phase extraction (SPE) which can be used to determine a broad range of pesticides in environmental samples (Kouzayha, 2012). The purpose of this study is to identify a simple and reliable method for analysis of 17 pesticides (14 Organochlorine and 3 Organophosphorus) in surface water. C18 a reversed phase Solid-Phase Extraction (SPE) cartridge has been used in this study. GC-ECD is used to determine and quantity the analytes. This method is applied to water samples of rice crops in both the organic and conventional fields from Ledang, Johor, Malaysia.

2. EXPERIMENTAL

Materials: Seventeen individual reference analytical grade standard namely aldrin, HexachlorocyclohexaneIsomers (α, β, γ, δ), Diazinon, Heptachlor, Malathion, Chloropyrifos, Heptachlor Epoxide, Endosulfan, Dichlorodiphenyl trichloroethane (DDT) family (4,4'-DDT 4,4'-DDE, 4-4'-DDD), Dieldrin, Endocrine and Endosulfan Sulphate were obtained from Sigma-Aldrich (St. Louise USA) and heptachlor epoxide was obtained from Supelco (Bellefonte, USA). All solvent used for sample extraction and analysis (methanol, n-hexane, acetone) were pesticide grade (Merck, Germany). Resprep C18 cartridge (6mL, 500mg) was purchased (Restek, USA) and using SPE (Manifold) for sample extraction. Organic free reagents water was obtained by using a Milli-QEasy pure Rodi system (Barnstead, USA).All pesticides were dissolved in acetone at 1000μg/mL concentration as the primary stock solution; the secondary stock solution of 100μg/mL was prepared from the primary solution. Then the mixed standard solution contained all the seventeenpesticides were prepared by pooling aliquots of the individual pure pesticide standard solution and then diluting with acetone. For GC-ECD analysis, a range of standard mixture stock solution containing 0.5μg/mL were prepared in acetone and stored at <4°C. Preparation of different concentration levels of stock solution is due to the sensitivity of the ECD detector. Standard solution of a mixture of pesticides was freshly prepared daily by volume dilution in acetone.

Instrument: Final determination of targeted compounds was achieved using GC-ECD, Varian CP3800 equipped with DB-5 capillary column (30m x 0.32μm x 1.0μm thickness), as outline in Table.1.

Table.1. Instrument setting for analysis of targeted compounds

Aspect	Setting	Aspect	Setting
Injection volume	1μL	Oven program	Initial 90°C to 170°C (ramped with 3.5°C min^{-1}), then up to 280°C (ramped with 5°C min^{-1}). Total runtime is 45.86 min.
Injection temperature	250°C	Flow rate	1.5 ml min^{-1} (99% purified nitrogen)
Detector temperature	300°C	Make up flow	25 mL

Extraction procedure: Pesticide mixture was spiked into organic-free reagent water 100µg/L concentration level. Cartridge $C_{18}$was primarily conditioned with a mixture of methanol and organic free reagent. Methanol is used as extraction solvent at different ratio. The cartridge was loaded with water sample at designed flow rate. Finally, the targeted compounds were eluted from C18 using acetone followed by n-hexane with the various ratios. Concentrated sample was placed under a nitrogen stream until dryness and reconstituted with 1 mL of n-hexane. The optimization method was based on previous work (Wang, 2009) with a few modifications on extraction procedure. Four variables namely: conditioning, sample volume, flow rate and the eluting ratio of targeted compounds were optimized in this study (Table.2).

Table.2. The matrix value of optimization in this study

Variables	Low	Middle	High
Solvent volume	1 mL	3 mL	5 mL
Sample volume	500 mL	750 mL	1000 mL
Eluting ratio (acetone: n-hexane)	2:8	5:5	8:2
Flow rate	0.5ml/min.	1.5ml/min.	3ml/min.

This procedure used one-variable at a time, with each factor was studied separately. The efficiency of the extraction, basically based on peak area of the analytes. Water samples were filtered under vacuum by using glass membrane, and then the water samples were processed using a solid phase extraction (SPE) technique. The cartridges (Resprep C18 6mL500mg) were conditioned with 5 ml of methanol followed by 5 ml of Milli-Q water. Water samples (1.0 L) were passed through the cartridge at a flow rate of 3.0 ml min^{-1} under N_2 pressure. Following extraction, the cartridges were eluted with 5.0 mL of acetone followed by 5.0 mL hexane. Samples were pre-concentrated under stream of nitrogen gas until dryness then it is followed by adding 1 mL n-hexane. Finally, 1µl was injected using GC-ECD.

Method Validation: The validation of the analytical method was performed by following parameters; Linearity, Limits of Detection (LODs), Limits of Quantification (LOQ), Recovery% and RSD%. Linearity was determined by constructing calibration curve with a standard solution in acetone, containing all the pesticides in the range of 50, 100, 200, 300, 400 and 500µg/L. Three injections were made at each of the six concentration levels. Recovery was performed by spiking the mixture into the organic free reagents water at concentrations 20µg/L. Determination of LODs and LOQs were according to guidelines (Shrivastava and Gupta, 2011). The RSD% of calculated from the triplicate analysis of each water samples.

Application on Real Samples: For real sample application, water samples were collected in 1L glass bottles from two different geographic points located in south Malaysia (Ledang) from Organic and Conventional fields. The study area is presented in Figure 1. Sampling sites were selected at both organic (S1) and conventional (S2) fields. Samplings were performed from October- 2015 to March- 2016. Water samples were filtered immediately through a Whatman GF/F filter 0.7 µm, extracted and analyzed as soon as possible according to the optimized procedures (Zhou, 1996).

Figure.1. Sample site of Ledang, Johor

3. RESULTS AND DISCUSSIONS

Selection of Pesticides: The seventeen pesticides selected, 14 OCPs and 3 OPPs were based on their being from different chemical families applicable to GC-ECD determination. The analyzed pesticides cover a wide range of compounds employed in agriculture particularly as insecticides. Under the optimized GC-ECD conditions; a baseline separation of the 17 targeted compounds was obtained. The identification of 14 OCPs and 3 OPPs were madeby standard solution retention time. The chromatogram of the 17 compounds in this study was shown in Figure.2.
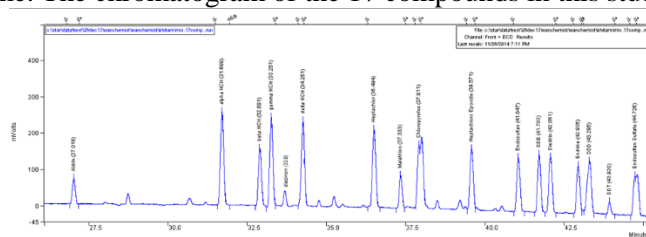

Figure.2. Chromatogram of 17 compounds by using GC-ECD

Solid Phase Extraction Procedure: For the optimization of the SPE-procedure, four parameters were investigated including sorbent selection, conditioning step, sample volume, flow rate and eluting ratio. Dependent on the aqueous matrix, different sample volume were extracted for detection in surface water, a sample volume 1000 mL was found to be sufficient to get $LOD_s \geq 0.01$ µg/L and for $LOQ_s \geq 0.037$ µg/L(Wille, 2011).

Optimization of Extraction:

Effect of cartridge conditioning: The first step in SPE cartridge is conditioning in which the solid sorbent is conditioned using appropriate solvent. This step is essential as it removes possible impurity the air present in the column and fills the void volume with solvent. The nature of the conditioning solvent depends on the type of the solid sorbent. Typically for reversed phase sorbent C18 Bond-Elute columns, methanol is usually utilized to pre-wet stationary phase, opens the hydrophobic chains to increase the effective surface area and used to provide better wettability for increasing the extraction efficiency of compounds (Sabik, 2000). Three series of organic-free reagent water were fortified with 1 mL spiked of the 17 targeted analytes. The SPE cartridges were conditioned with 1, 3 and 5 mL of methanol before extracting analytes from the water sample. Figure 3 shows that 5 mL of methanol is the best volume for conditioning due to the higher recovery.
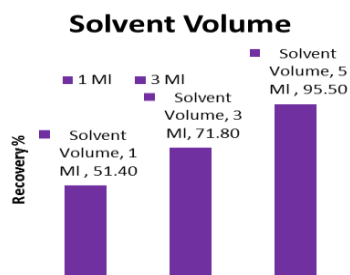

Figure.3. Effect of solvent volume

Effect of sample volume: Series of experiments were conducted to verify the optimum volume of sample for extraction. There are a series of sample volume 500, 750 and 1000 mL of organic-free reagents water were fortified with 1 mL (0.1 mg/L) spiked of 17 targeted analytes. In practical environmental analysis, the need to determine trace amounts of organic compounds has led to an increase of the sample volume from a few milliliters to hundreds of milliliters and even liters (Kouzayha, 2011) and consequently breakthrough becomes more of a concern. Some authors have shown experimentally that the retention and recovery of analytes depend on the sample volume that flows through the SPE cartridge (Liska and Kuthan, 1990). For sample volume, the range depends on the concentration of analytes in water, the quantity of adsorbent in the SPE cartridge, and the detector sensitivity. In this sample volume of 1 L is chosen to extract sufficient pesticide residues often occur at trace concentration level (Johnson, 1991). The recoveries for all compounds were satisfactory with 1L water samples. As shown in Figure.4, a very low recovery of pesticides (16-20%) was observed using 500 and 750 mL of the water sample as compared to 1 L sample volume (Molto, 1991).
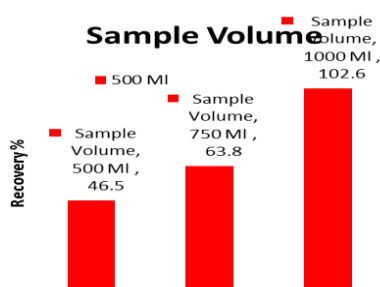

Figure.4. Effect of sample volume

Effect of eluting solvents: The elution strength of the organic solvent depends on the type of sorbent used, so different elution solvents and procedures were evaluated for theC18 cartridge. The organic solvents considered in elution procedures were from various polarities including n-hexane and acetone. For traditional elution, a large volume of the elution phase is percolated slowly on SPE cartridge to ensure desorption of analytes from the stationary phase. The optimum solvents ratio as eluent were found to be (1:1) (acetone: n-hexane) (Figure.5). This yield high recoveries, as the mixture of solvent overcome the interactions between sorbent and C18, thus, OCPs, OPPs were allowed to pass through the sorbent. Retention of the analyte under reversed phase is primarily because of the polar functionality of analyte and sorbent surface. This is due to solvent disrupting the binding mechanism. The solid phase extraction method was chosen for subsequent monitoring studies of these pesticide residues in water samples (Biziuk, 2006; Cho, 2007; Kouzayha, 2011).
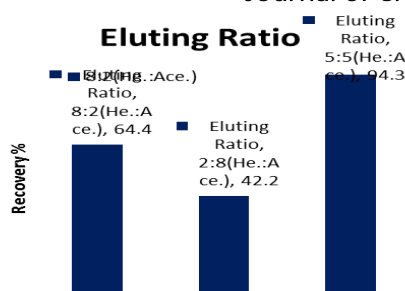
Figure.5. Effect of eluting ratio

Effect of eluting Flow Rate: The effect of the elution process was studied by changing the flowrate between 0.5 and 3.0 mL/min. So pesticides were eluted throughout this eluent with no carry-over (i.e., elution was complete) with an eluent volume. The eluting flow rate of the sample through the column during pre-concentration step had a tiny effect on the adsorption efficiency over the studied range (0.5-3.0)mL/min. So 3.0 mL/min is chosen as the optimum flowrate due to the adsorption efficiency of analyte (Ballesteros and Parrado, 2004) as shown in Figure 6.
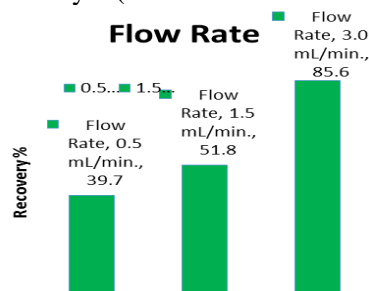

Figure.6. Effect of flow rate

Validation parameters of the analytical methodology: The performance of the optimized SPE-GC-ECD analysis method was validated to evaluate the linearity, recovery, precision and limits of (LOD) and (LOQ). The results are listed in Table.6. Linearity was determined by the instrumental response. The range of concentration considered was 5 - 500 $\mu g.L^{-1}$ at six concentration levels (50, 100, 200, 300, 400 and 500) $\mu g.L^{-1}$. The calibration curves were plotted using a least-square regression analysis. Each compound showed good linearity for the GC-ECD analysis in the studied working range, with a correlation coefficient ($R^2 \geq 0.992$).

Recovery was performed by spiking the mixture into the organic free reagents water 20μg/L concentration. Determination of LODs and LOQs were by guidelines (Shrivastava and Gupta, 2011). For this purpose, seven independent analyses of water were spiked with pesticides at the level of 20μg/L. LODs and LOQs were calculated from the standard deviation of these determinations based on the equation 1 and 2 below:

$$LOD = 3 \times SD \qquad\qquad\qquad ….(1)$$

$$LOQ = 10 \times SD \qquad\qquad\qquad …..(2)$$

Analysis of Real Samples: Table.7, shows the OCP$_S$ and OPP$_S$ compound residue concentration in water samples taken from irrigation canals in paddy fields. In organic field, the average concentration for OCP$_S$ ranges from not detected(ND) to 0.662μg/L and from ND-0.1μg/L for OPP$_S$. For Conventional fieldsthe average concentration for OCP$_S$ ranges from 0.059to 0.526 μg/L and OPP$_S$ from 0.081 to 0.109μg/L.

Some ofpesticide residuesin water sample are exceeded the European Economic Commission (EEC) safe limit (Directive 98/83/EC)($\leq 0.1\mu g$ /L for any pesticide or $\leq 0.5\mu g$ /L for total pesticides)which are α-HCH, γ-HCH, heptachlor, heptachlor epoxide, endosulfan, DDE, dieldrin and DDD from organic field and all OCP$_S$ except DDT from conventional field.

In addition, high frequency of detections were observed for are α-HCH, γ-HCH, heptachlor epoxide, DDE and dieldrin from both organic and conventional field indicate that these pesticides were highly used in the study area.DDT was much less than DDD & DDE indicate the historical of new used of DDT, there was no indication of new used of DDT.

The presence of these pesticides can be attributed to their extensive usage during the 1950s and 1960s, especially in the rice fields. These pesticides particularly OCPs degrade very slowly and accumulate in the soil of the rice fields and are subsequently leached out into the aquatic system of the surrounding area (Tan, 1992). This result showed that pesticides are still being used in the paddy fields in the areas. Although Organochlorine pesticide has been banned since 2008 in many countries including Malaysia for use in crop cultivation, they may be used illegally in developing countries like Malaysia because of the low cost, effectiveness and availability (Azhani, 2012).

Test of variance (ANOVA) was performed on the results of pesticide residues from organic and conventional fields. The result showed that the concentration of the pesticide residues in water samples from organic fields were

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not significantly different from those from conventional fields (p>0.05) in spite of the non-use of pesticides in the organic fields. This is due to rainfall, natural processes such as leaching or the irrigation system that enhance the transfer of pesticide residues from conventional fields to the organic fields during planting season (Arjmandi, 2010). Water flow in the paddy field also affected the concentration of the pesticides. The concentration of pesticides in paddy plants at the water inlet area for both fields own practice areas were the lowest compared to that in mid-field and outlet areas. This is because the clean water from the inlet areas diluted the pesticide residues in the paddy plants. The proceeding with the nearness of these chemicals in the paddy field and the general environment in Asia is most likely because of a combination of the persistent character of OCP_S compounds and their continuing, illegal, use by farmers.

The partitioning of OCP_S insecticides in water, sediment, and fish in the paddy field environment, attributed to the solubility characteristics associated with these chemicals is illustrated in Malaysia (Abdullah, 1997). This is quite reasonable by the similar physiochemical characteristics in their irrigation waters and similarity of the climatic conditions in the studied area (Ballesteros and Parrado, 2004). The presence of these pesticides can be attributed to their wide usage during the 1950s and 1960s, especially in the rice fields. These Organochlorine pesticides degrade very slowly and accumulate in the soil of the rice fields and are subsequently leached out into the aquatic system of the surrounding areas. Currently, organophosphorus insecticides are used because most of the Organochlorine insecticides have been banned due to their toxicity, persistence, and bioaccumulation in the environment (Font, 1993; Tan, 1992). These insecticides are transported into the ground water through leaching, channeling, direct spillage and wind drift.

Table.3. Linearity, R^2, (LODs), (LOQs), Recovery% and RSD% results obtained for all compounds

Compounds	R^2	Recovery %		RSD %		LODs	LOQs
		Net Recovery	Overall Recovery	Net RSD	Overall RSD	μg/L	μg/L
Aldrin	0.998	97.5 101	99.25	1.66 2.79	2.22	0.01	0.035
Alpha-HCH	0.996	99.8 102.9	101.35	0.56 1.00	0.78	0.02	0.067
Beta-HCH	0.998	108.1 107.60	107.85	4.28 3.11	3.70	0.028	0.094
Gamma-HCH	0.992	102.5 110.30	106.4	0.72 1.48	1.10	0.031	0.105
Diazinon	0.997	77.2 71.3	74.25	4.86 3.29	4.07	0.028	0.095
Delta-HCH	0.996	111.2 121.60	116.4	2.50 3.20	2.85	0.058	0.195
Heptachlor	0.995	88.6 74.10	81.35	0.95 3.87	2.41	0.029	0.097
Malathion	0.998	74.5 77.40	75.95	4.60 2.16	3.38	0.041	0.138
Chloropyrifos	0.997	81.8 80.70	81.25	0.06 0.71	0.39	0.021	0.07
Heptachlor Epoxide	0.997	95.7 88.70	92.2	1.62 4.66	3.14	0.079	0.263
Endosulfan	0.998	103.6 105.80	104.7	0.52 2.81	1.67	0.071	0.23
DDE	0.998	87.5 82.30	84.9	2.21 1.18	1.70	0.081	0.272
Dieldrin	0.996	102 105.30	103.65	1.89 1.13	1.51	0.021	0.07
Endrine	0.995	87 79.00	83	2.56 2.36	2.46	0.074	0.249
DDD	0.994	94 81.30	87.65	2.19 1.04	1.61	0.088	0.29
DDT	0.996	105.3 117.00	111.15	1.64 3.31	2.47	0.02	0.067
Endosulfan Sulfate	0.998	99.8 105.70	102.75	2.13 7.04	4.59	0.018	0.06

*LODs= Limit of Detection, *LOQs= Limit of Quantification,*RSD= Relative Standard Deviation

Table.4. Concentration levels of pesticide residues in water sample from sampling trips

Compounds	Organic Field			Conventional Field		
	Mean (µg/L)±SD	Range (µg/L)	FD (%)	Mean (µg/L) ±SD	Range (µg/L)	FD (%)
Aldrin	0.054± 0.004	ND-0.143	25	0.103± 0.004	ND-0.153	40
Alpha HCH	0.129± 0.006	ND-0.187	93	0.176± 0.007	ND-0.192	95
Beta HCH	0.073±0.005	ND-0.200	25	0.115± 0.025	ND-0.227	30
Gamma HCH	0.153± 0.024	ND-0.224	75	0.165± 0.005	ND-0.235	65
Diazinon	0.029± 0.007	ND-0.081	20	0.077± 0.012	ND-0.105	75
Delta HCH	0.094± 0.021	ND-0.224	40	0.165± 0.004	ND-0.268	40
Heptachlor	0.127± 0.006	ND-0.218	45	0.179± 0.003	ND-0.231	60
Malathion	0.030± 0.007	ND-0.101	20	0.066± 0.019	ND-0.109	60
Chloropyrifos	0.022± 0.009	ND-0.058	40	0.045± 0.025	ND-0.081	50
Heptachlor Epoxide	0.421± 0.015	ND-0.552	87	0.526± 0.017	ND-0.559	90
Endosulfan	0.251± 0.042	ND-0.370	70	0.355± 0.028	ND-0.389	95
DDE	0.152± 0.020	ND-0.255	93	0.195± 0.021	0.110-0.296	100
Dieldrin	0.167± 0.043	ND-0.270	81	0.254± 0.018	ND-0.274	95
Endrine	ND	ND	ND	0.386± 0.009	ND-0.663	30
DDD	0.452± 0.088	ND-0.662	55	0.497± 0.068	ND-0.695	65
DDT	0.034± 0.010	ND-0.120	20	0.059± 0.001	ND-0.139	25
Endosulfan Sulphate	ND	ND	ND	0.107± 0.003	ND-0.146	35

*ND= Not Detected,*FD=Frequency of Detection, *SD = Standard Deviation

4. CONCLUSION

In conclusion, the use of solid-phase extraction provides a rapid, efficient and reproducible method for the simultaneous determination of various pesticides in waters. The two-step extraction and concentration procedure minimizes residue losses and contamination problems. The simplicity of the analysis is complemented by good GC-ECD results. The widespread occurrence of pesticide residues in the natural waters of the paddy fields in Ledang, Malaysia indicates pollution as a result of agricultural activity. Although pesticide residues were detected in water in the paddy areas were mostly still within the permissible limits, there is a need to monitor their presence in order to ensure their safety to consumers.

5. ACKNOWLEDGMENT

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