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ANALYSIS OF ORGANOCHLORINE PESTICIDES IN THE HINDON RIVER WATER, INDIA

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Abstract

Pesticides are notorious contaminants due to their toxic and carcinogenic nature and, unfortunately, are distributed globally in air, water, sediment, soil and foods even to the places where they have not been used before. SPE and GC methods were developed to analyze organochlorine pesticides. The optimization was achieved by varying SPE conditions such as different pH of wastewater, flow rates of waste water, flow rates of solvents and different eluting solvents. Also optimization of GC conditions was obtained by using different columns, gases with various flow rates and temperatures. The optimized method was applied to analyze organochlorine pesticides in the Hindon river, India. The observed pesticides were α-BHC, β-BHC, γ-BHC, aldrin, endosulfan, DDE, DDD and methoxychlor of the concentration ranges from 8.1 to 26.8 μg/L. Extraction recoveries were 89.5, 90.0, 90.0, 92.3, 97.0, 97.9, 97.0 and 98.1 percentages for α-BHC, β-BHC, γ-BHC, aldrin, endosulfan, DDE, DDD and methoxychlor, respectively. It has been observed that the concentrations of pesticides increased from Behreki to Mohan Nagar sampling points, which may be due to the increase of pesticide pollution from agriculture, domestic and industrial activities. The concentration of α-BHC was found to be highest 26.8 μg/L. Validation of the developed methodology was carried out by using Microsoft Excel program.

Keywords: Gas Chromatography, Solid Phase Extraction, Hindon River, Organochlorine Pesticides.

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Introduction

Among various environmental pollutants organochlorine pesticides (OCPs) raised anxiety in the society due to their toxic nature, carcinogenicity and long existance in the environment. Generally, OCPs show strong hydrophobic nature, which results in their accumulation in fatty tissues, and as consequences it becomes carcinogenic and endocrine disruptor in mammals [1,2]. Therefore, it is true that their presence in the environment even at nano/pico level can cause serious damage to human health. Rivers in India are one of the important sources of water supply and, unfortunately, are exposed to pesticides pollution. Therefore, it is very important to monitor water quality; in terms of the presence of pesticides; prior to supply to the society. In view of this, the detection technique with low detection limit is very important. In this area solid phase extraction and gas chromatography are popular techniques and some workers used these to monitor organochlorine pesticides in different matrices [3-15]. In view of these points, attempts have been made to apply SPE and GC technique for the analyses of organochlorine pesticides in water of the Hindon river; an important river of Northern India and the results are discussed herein.

Experimental

Chemicals and Reagents

The OCPs (α-BHC, β-BHC, γ-BHC, aldrin, endosulfan, DDE, DDD and methoxychloro) were obtained from Sigma chemical Co., USA. The standard solutions (1.0 μg/mL) of individual and the mixture of organochlorine pesticides were prepared in hexane. The water used was prepared by using a Millipore Milli-Q water purification

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system (Bedford, M.A., U.S.A.). Sodium hydroxide, methanol, triethylamine, ethanol, ethylacetate, acetic acid, acetone, and hexane were of A.R. grade and were purchased from Merck India. Nitrogen gas of grade A was received from Goel Gases, Ghaziabad, India.

Instruments used

pH meter of Hach, USA was used to measure pH of the solutions. Millipore Milli-Q (Bedford, MA, USA.) was used for deionised water. 1.0 mL C_{18} cartridges (Sep-Pak Vac C_{18}) were purchased from Waters, USA.

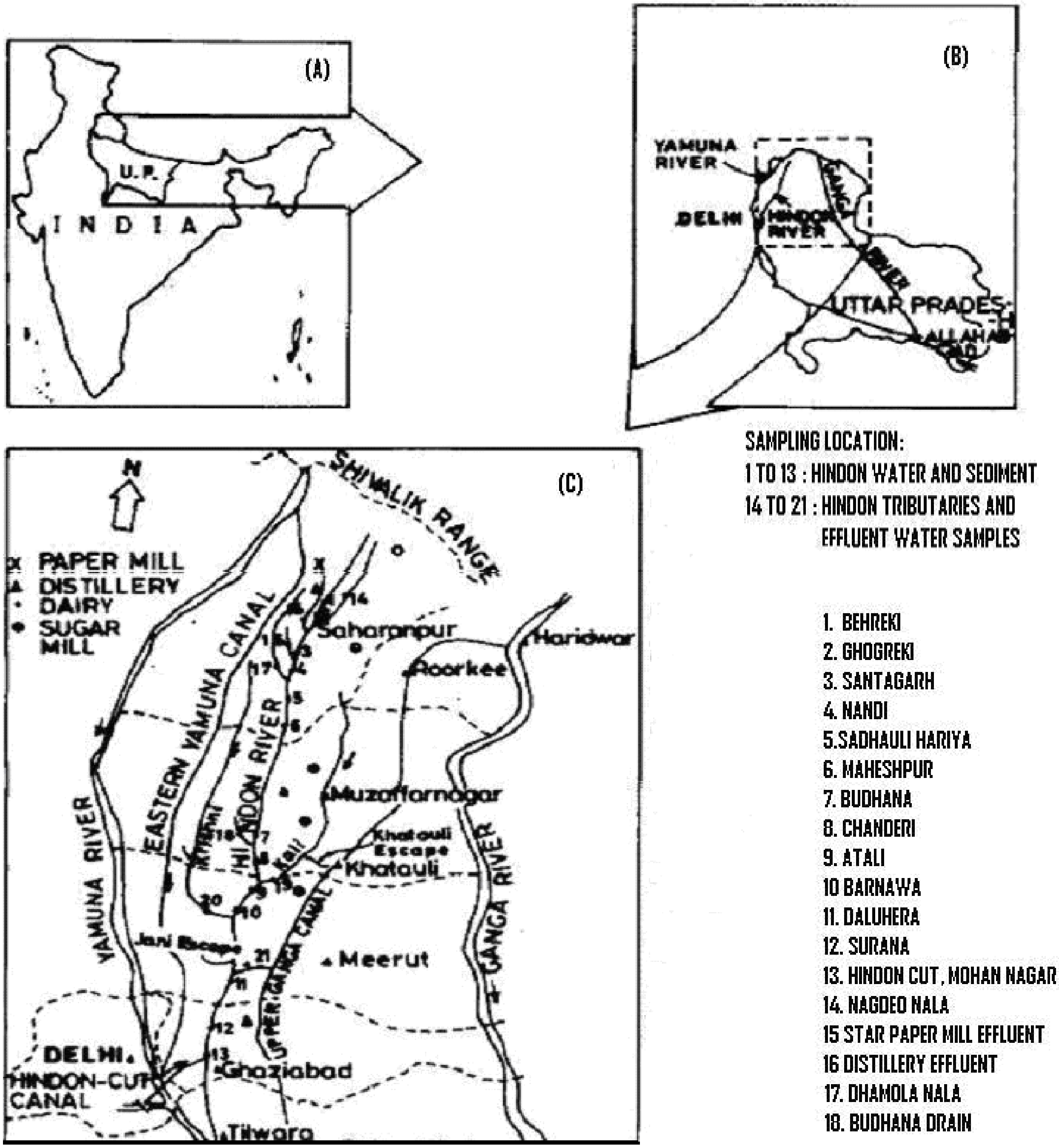

Figure 1. Location of sampling points

The vacuum assembly was homemade and the vacuum was generated by the homemade vacuum pump. The analysis of the reported OCPs after extraction was carried out by gas chromatography. Gas chromatograph used was of Nucon, India, with model 5700. The column used was Equity-5 (30 m x 0.25 mm, IP 0.25 μm) and obtained from Sigma Chemical Co., USA.

Sample Collection

For the present studies, grab water samples were collected from the Hindon river, at different 13 locations (Figure 1). These sample water was collected in the pre-washed glass containers closed with Teflon lined caps. Water samples for pesticides were preserved at sites by adding 10 mL of chloroform and stored immediately at 4 ˚C till further experiments.

Solid Phase Extraction (SPE)

SPE methodology was developed by spiking of 1.0 mL of organochlorine pesticides mixture of 1.0 mg/mL concentration each (in methanol-water) in 999.0 mL tap water. This mixture was shaken for about 30 minutes and pH was adjusted to (2 to 10.0) with concentrated sodium hydroxide and hydrochloric acid solutions. The spiked water sample was kept at room temperature overnight. C18 Cartridge was pre-conditioned by using methanol (1.0 mL) followed by water (1 mL). After equilibrium, 1.0 L of the spiked water was passed through this cartridge at (10.0 to 100.0) mL/min flow rates. Cartridge was washed with 2.0 mL of deionized water and then the elution of OCPs was carried out by using hexane at different flow rates (0.2 to 1.0 mL/min) three times. Three fraction of eluted hexane were combined together. Besides, the elution was also tried with dichloromethane, hexane, methanol, ethanol and acetone. This methodology was applied to the natural conditions by replacing tap water by Hindon river water and the results were compared. The developed SPE procedure was applied for the extraction of OCPs from water samples obtained from the Hindon river. Waste water samples filtered through Whatman filter paper No. 24 and the extraction procedure was applied as described above.

Gas Chromatography (GC)

Gas Chromatographic method was developed for analysis of α-BHC, β-BHC, γ-BHC, aldrin, endosulfan, DDE, DDD and methoxychlor pesticides. The separations of these pesticides in standard solutions were shown in Figure 2. It is clear from this chromatogram that the shapes of the peaks are sharp indicating a good separation. The retention times of each organochlorine pesticide were identified by running gas chromatograph for individual pesticides under the reported identical conditions of the experiments. The capacity factor and the separation factor were calculated. The chromatographic conditions were optimized by varying the temperatures of injection, column and detector. Besides, flow rates of the carrier gas were also optimized. As a result of extensive experimentation, the best chromatographic conditions were developed and reported herein.

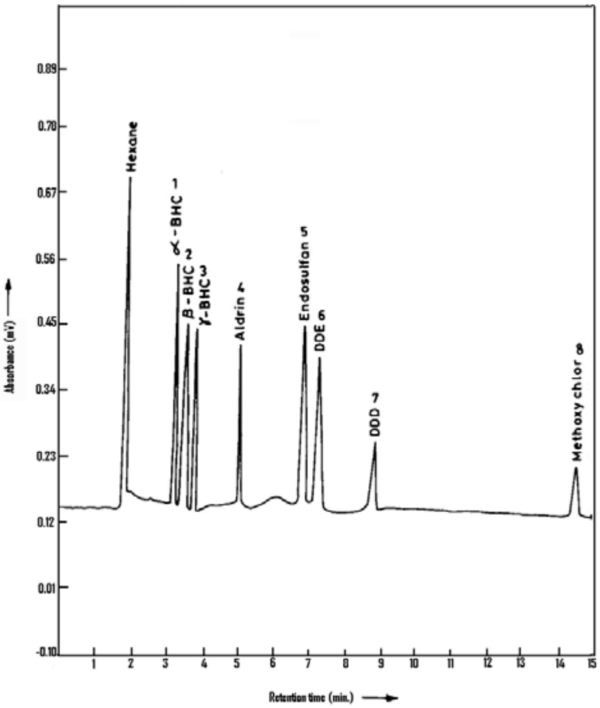

Figure 2. GC Chromatograms of organochlorine pesticides in standard solution.

Results and Discussion

As a result of the above discussed SPE experiments, the recoveries of pesticides from tap water samples were 89 to 99% by using the above describe SPE method. The recoveries of organochlorine pesticides studied are given in Table 1.

Table 1. Percentage Recoveries of Organochlorine Pesticides in Standard Solutions and River Water Samples by using Solid Phase Extraction (SPE) Method.

Pesticides	% Recovery	
	Standard Solutions	River Water Samples
α-BHC	89.8	89.5
β-BHC	90.2	90.0
γ-BHC	90.0	90.0
Aldrin	98.0	92.3
Endosulfan	97.5	97.0
DDD	98.2	97.9
DDE	97.0	97.0
Methoxychlor	98.5	98.1

It is clear from this table that α-BHC, β-BHC, γ-BHC, aldrin, endosulfan, DDE, DDD and methoxychlor are recovered 89.8, 90.2, 90.0, 98.0, 97.5, 98.2, 97.0 and 98.5 percents, respectively, indicating a good efficiency of solid phase extraction. The separations of these pesticides in standard solutions are shown in Figure 2. The chromatogram has no extra peaks or noise, which is confirmation of the best working of solid phase extraction method. It means that the solid phase extraction is unique and specific in nature and capable to extract only interested pesticides under the reported experimental conditions. Gas chromatographic values i.e. retention times (tr), capacity (k) and separation (α) factors for the separated chlorinated pesticides in standard solutions are given in Table 2. It is clear from this table that the values of tr, k and α are in the range of 1.5 to 9.00, 0.78 to 7.0 and 1.04 to 8.95, respectively. All these values indicate a good separation and identification of organochlorine pesticides by developed GC method.

Table 2. Retention time (tr), capacity factor (k) and separation factors (a) of Organochlorine Pesticides in Standard Solutions.

Pesticides	Retention time (t_R)	Capacity factor (k)	Separation factor (α)
α - BHC	3.4	0.78	1.28
β – BHC	3.6	1.0	1.11
γ - BHC	3.8	1.11	1.60
Aldrin	5.4	1.78	1.50
Endosulfan	6.8	2.67	1.04
DDD	7.4	2.78	1.08
DDE	9.0	3.00	2.33
Methoxychlor	1.5	7.00	8.95

t_0= 1.8 minutes

Optimization
To achieve maximum extraction, SPE conditions were optimized and the parameters optimized are flow rate of water, pH of water, flow rate of eluting solvent and use of different solvents, which are discussed below.

Effect of Water Flow Rate
Normally, high flow and low flow rates result into poor and good percentage recoveries. Therefore, optimization was achieved by using 10, 25, 50, 75 and 100 mL/min. flow rates in these experiments. The results of these findings are shown in Figure 3. It is clear from this figure that maximum percentage recoveries were obtained at 10 mL/min. The percentage recoveries of organochlorine pesticides were in the order of 10 > 25 > 50 > 75 > 100 mL/min. Of course, 10 mL/min. gave maximum recoveries but took longer time and there was not much difference in percentage recoveries between and 10 and 50 mL/min. Therefore, 50 mL/min. was selected as the best flow rate.

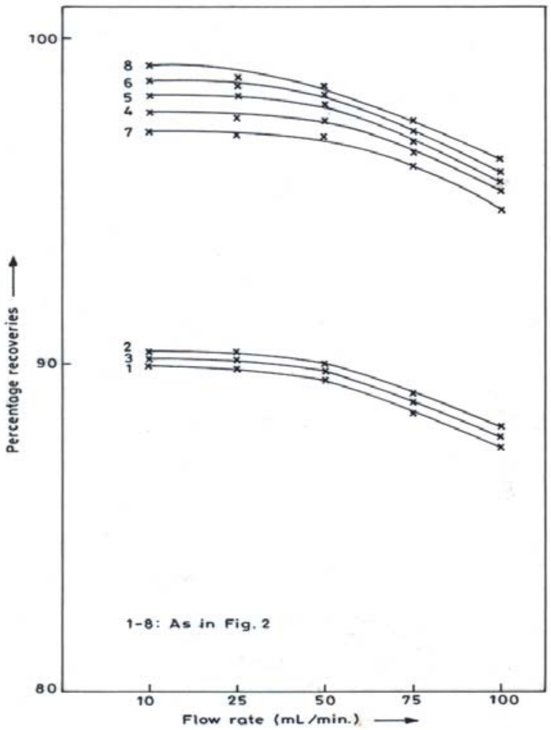

Figure 3. Effect of water flow rate on percent recovery OCPs.

Effect of pH
pH of water plays an important role in SPE of various organic pollutants. The percentage recoveries of the compounds depend on the adsorption process, which is controlled by pH of water up to a good extent. The effect of pH was studied using pH from 2.0 to 10.0. The results of pH variation on percent extraction are shown in Figure 4.

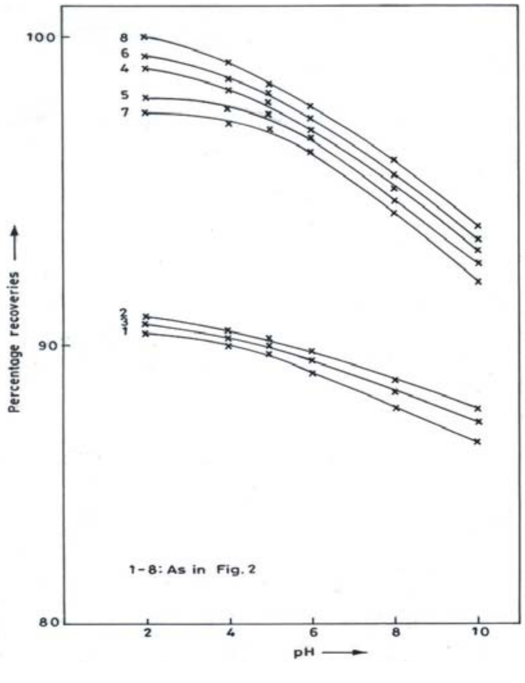

Figure 4. Effect of pH on percent recovery OCPs.

It is clear from this figure that extraction percentages are higher for all OCPs at low pHs. The maximum extraction of methoxychlor was 99.9% at pH 2.0 while it decreases to 93.9 at pH 10.0. Similar nature was observed for other pesticides too. This sort of behavior can be explained on the basis of the interactions between OCPs and C18 material of solid phase cartridge. Under acidic conditions, all pesticides have positive charges due to the presence of hydrogen ions. The positive charges of these molecules bind them to C_{18} material of solid phase cartridge through electrostatic forces of attraction. In spite of this, pH 5.0 was selected through out the experiments, as the percentage recoveries are quite good; moreover, it is the pH of some natural water resources.

Effect of Eluting Solvent Flow Rates

The percentage recoveries of the compounds are also governed by the flow rate of the eluting solvent through C18 cartridge. Generally, high flow rate results into poor percentage recoveries while low flow rate is used for high recoveries. Four flow rates i.e. 0.2, 0.5, 0.8 and 1.0 mL/min. were tried for the extraction of organochlorine pesticides. The results of this set of experiments are shown in Figure 5.

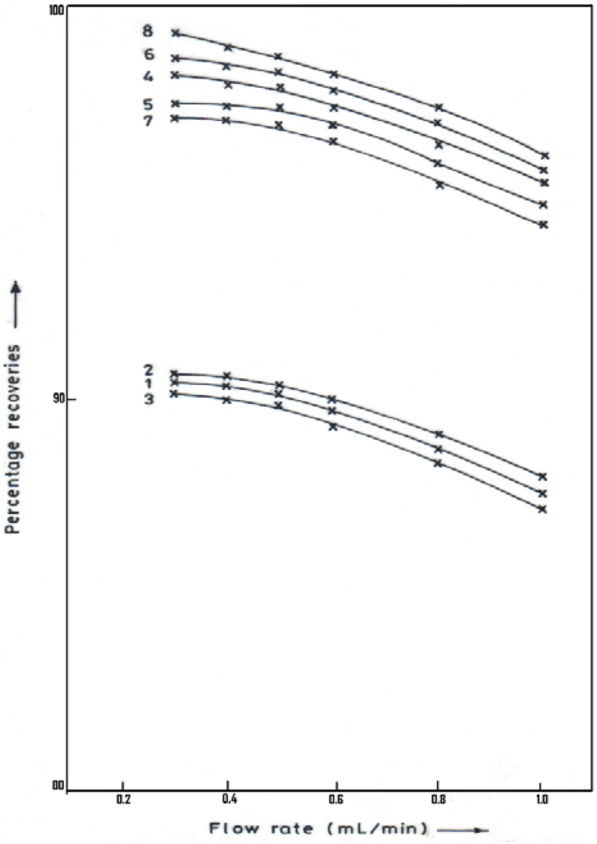

Figure 5. Effect of flow rate of eluting solvent on percent recovery OCPs.

It is clear from this figure that the maximum percentage recoveries were obtained at 0.2 mL/min. while lower percentage recoveries were observed at 1.0 mL/min. The percentage recoveries of OCPs were in the order of 0.2 > 0.5 > 0.8 > 1.0 mL/min. The elution of OCPs from C_{18} cartridge material is occurred due to desorption of OCPs. Therefore, low flow rate provides maximum time to pass through solid phase extraction resulting into high desorption of OCPs from C_{18} material. Contrarily, high flow rate does not give the sufficient time to desorb organochlorine pesticides from C_{18} material, which resulted into poor percentage recoveries. But due to convenience and economy point of view 0.5 mL/min. was used as the optimized flow rate.

Effect of Eluting Solvents

In addition to the above-cited parameters, the optimization of SPE was also tested by using different eluting solvents. The other eluting solvents tried were dichloromethane, methanol, ethanol and acetone. The percentage recoveries of the reported organochlorine pesticides using above cited solvents are given in Figure 6. It is clear from this figure that the percentage recoveries are in the order of dichloromethane > hexane > methanol > ethanol > acetone. Dichloromethane is the best eluting solvent but it could not be injected on GC machine having ECD detector. Therefore, hexane was used as the best eluting solvent through out this study.

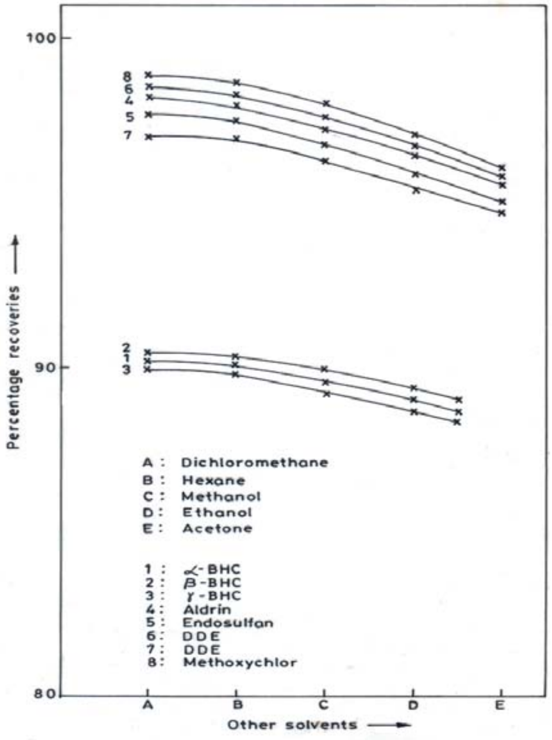

Figure 6. Effect of other solvents on percent recovery of OCPs.

Validation of the Method

The validation of solid phase extraction and gas chromatography methodologies was confirmed by carrying out these experiments five times under the identical experimental conditions. The regression analysis was carried out by using Microsoft Excel program. The values of standard deviation obtained were ± 0.13 to ± 0.15 and ± 0.20 to ± 0.23 for GC and SPE methods, respectively. The values of the correlation coefficients (R^2) were 0.9999 to 0.9999 and 0.9998 to 0.9999 for GC and SPE methods, respectively. The confidence levels were 98.5 to 99.1 % and 97.4 to 98.3 % for SPE and GC methods, respectively. These values of validation parameters indicate good reproducibility of developed SPE and GC methodologies.

Applications of SPE and GC Method in the Hindon River

The developed SPE and GC methodologies were applied for the analysis of organochlorine pesticides in the Hindon river. For this purpose, river water samples were collected from the Hindon river at 13 different sites (Figure 1). The values of concentration of the organochlorine pesticides observed are given in Table 3. A perusal of Table 3 indicates that analysis of organochlorine pesticides have been carried out for water samples collected from Behreki, Ghogreki, Santagarh, Nandi, Sadhauli Hariya, Maheshpur, Budhana, Chandheri, Atali, Barnawa, Daluhera, Surana and Mohan Nagar sampling stations of Ghaziabad, India. The maximum concentration of methoxychlor was found at Mohan Nagar while the minimum concentration observed of γ- BHC was at Maheshpur. However, Aldrin, DDD and DDE pesticides were absent in the Hindon river water. The percentage recoveries of OCPs from river water samples were 89.5, 90.0, 90.0, 92.3, 97.0, 97.9, 97.0 and 98.1 for α-BHC, β-BHC, γ-BHC, aldrin, endosulfan, DDE, DDD and methoxychlor, respectively.

The slightly lower values of recoveries in the river water may be due to the presence of other impurities in the river water. For the convenience of readers, GC chromatograms of pesticides in real river water sample at Mohan Nagar, Ghaziabad, U.P., India) are shown in Figure 7. It is clear from this figure that all the reported pesticides are base line separated under the reported experimental conditions. The shapes of the peaks are sharp indicating a good separation.

Conclusions

The developed SPE and GC methods were used for the separation, identification, and quantification of organochlorine pesticides in the Hindon river water. The values reported of pesticides in the Hindon river water indicates that the river is polluted and water is not fit for drinking, recreation, washing purposes and other purposes. Besides, these methodologies are rapid, selective and reproducible. The percentage extractions of organochlorine pesticides are quite good. Therefore, these methods can be used for the analysis of organochlorine pesticides in waste, surface, ground and mineral water samples.

Table 3. Concentrations of organochlorine pesticides in the Hindon river (µg/L).

Sampling sites	α-BHC	β-BHC	γ-BHC	Aldrin	Endo-sulfan	DDD	DDE	Meto
Behreki	10.5	10.2	10.0	ND	12.0	ND	ND	12.0
Ghogreki	10.0	9.0	8.9	ND	10.0	ND	ND	10.0
Santagarh	10.0	9.0	8.8	ND	10.0	ND	ND	9.8
Nandi	10.0	9.0	8.5	ND	9.8	ND	ND	9.5
Sadhauli Hariya	10.0	8.7	8.3	ND	9.4	ND	ND	9.0
Maheshpur	10.0	8.5	8.1	ND	9.2	ND	ND	8.8
Budhana	10.7	9.0	9.0	ND	10.2	ND	ND	10.0
Chandheri	10.8	9.6	9.6	ND	10.5	ND	ND	10.6
Atali	23.2	20.2	20.0	ND	22.8	ND	ND	22.9
Barnawa	23.5	20.6	20.8	ND	23.0	ND	ND	23.6
Daluhera	23.8	20.7	21.0	ND	23.0	ND	ND	23.7
Surana	24.9	20.9	20.8	ND	23.0	ND	ND	23.6
MohanNagar	26.8	22.8	23.0	ND	23.5	ND	ND	24.9

ND: Not Detected, Meto.: Methoxychlor.

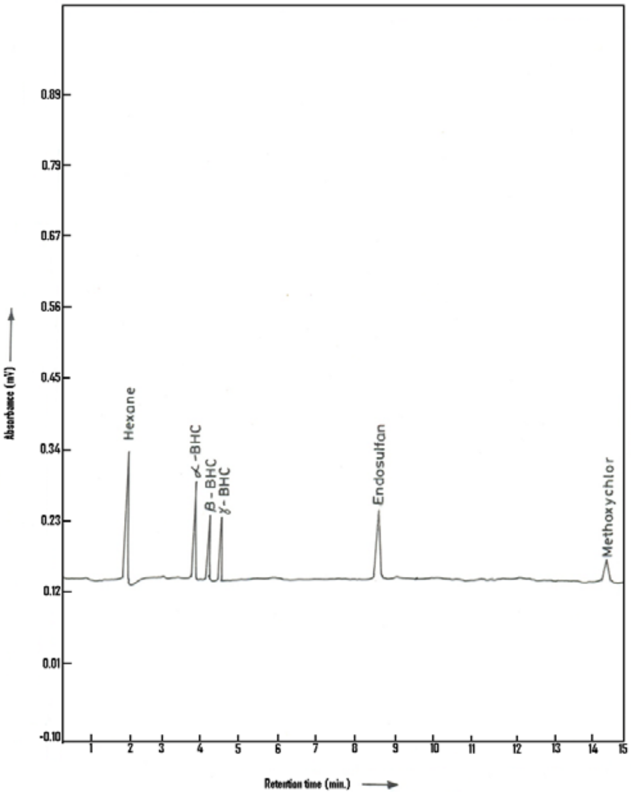

Figure 7. GC chromatograms of OCPs in Hindon River water samples.

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