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Application of Chromatographic Techniques for Determination of Insecticides and Pesticides in Waste Water Used for Irrigation

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Abstract: The frequent use of synthetic pesticides and their subsequent fate in the environment poses a major health implication to the population. Determination of these residues is necessary to ascertain human exposure as a result of contaminated dietary intake. In the present study chromatographic techniques, thin layer chromatography and high pressure liquid chromatography, have been employed for qualitative and quantitative determination of selected pesticides and insecticides in waste water of drains which is used to fulfill agricultural and irrigation needs of the area. Water samples collected from Chota Ravi Drain, Gulshan-e-Ravi Drain, Saggian Drain, Hudiara Drain, Mian Mir Drain and Kharak Drain were analyzed. The results showed that Hudiara Drain, Mian Mir Drain and Kharak Drain were severely contaminated with insecticide Deltomethrinin at concentration of 0.91 mg/l, 1.75 mg/l and 2.05 mg/l, respectively and pesticide Crypermethrin in concentration of 7.17 mg/l, 6.23 mg/l and 0.47 mg/l, while Chorophyrophos concentration was 441.02 mg/l, 383.3 mg/l and 29.04 mg/l, respectively. The presence of these persistent agrochemicals in drain is disturbing as throughout the year farmers' use this water for irrigation and these agrochemicals has the potential to enter the food chain thus adversely effecting environment and human health.

Key words: High Performance Liquid Chromatography · Thin Layer Chromatography · Pesticides · Waste Water

INTRODUCTION

As the world progresses towards newer chemicals and more advanced technology, it often fails to account for the side effects of advancement. Pesticides and insecticides are examples of synthetic chemicals which have proven to be both a blessing, in view of their potential to address food security issues, as well as a curse, due to their persistence and bio magnification along food chain inflicting adverse health effects on biotic life. It can be argued that humans, animals, marine life; nearly all living beings today stand affected by the harmful toxins produced by these synthetic chemicals. This class of compounds is toxic substances applied to control diverse insects and pests either by killing them or preventing them to cause destruction in crops.

A range of pesticides have been used extensively in agricultural processes in pre and post-harvest processes. Bioaccumulation and bio magnification of these in higher trophic levels via food chain poses a serious health and environmental concern [1]. Several researches have been conducted on the bioaccumulation of pesticides and the associated health effects. Organochlorine and polychlorinated biphenyl pesticides are known endocrine disruptor [2,3].These compounds, classified on the basis of their basic chemistry, toxicological action and mode of penetration [3,4], destroy pests and insects by affecting their nervous system, acting as endotoxins and regulating their growth mechanism, inflicting genotoxicity and hormonal disruptions[1]as well as displaying high acute toxicity and mutagenic and carcinogenic effects [3]. The extensive and indiscriminate application of insecticides and pesticides also results in the contamination of surface water, ground water, soil and crop [5]. As reported, consumption of such contaminated water and food stuff causes adverse human health effects which have been linked to the presence and application of pesticide residues contaminated water and soil thereby

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resulting in contamination of agriculture produce/crops (vegetables and fruits). Moreover, long term occupational exposure to pesticides can affect the health of farmers. An econometric study showed direct chronic health effects due to prolonged pesticide exposure, while population living close to agricultural fields as well as general public, through intake of contaminated food/ drinks, are also at a risk of potential exposure [6].

Several techniques can be employed to determine pesticides and insecticides residues in water samples. Use of thin layer chromatography (TLC), High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) has been reported in literature [7, 8]. The current study was conducted firstly to test the applicability of TLC detection methods for screening of pesticide/ insecticides residues by selecting the most appropriate solvent system for detection by TLC and secondly for the determination of the precise concentration of identified pesticides via HPLC analysis.

The six drains under study are highly polluted due to discharge of industrial effluents and pesticides from the nearby agricultural fields. The water from these drains ultimately falls into River Ravi and this water is then again used by the farmers for irrigation and other purposes. Thus, the over use of toxic water in agriculture has raised some plausible concerns such as the presence of toxins and pesticides in water that cause unpleasant and unsafe taste and odor, adverse effects on aquatic life and detrimental effects on farmer's health and the surrounding environment.

Methodology

Sampling: Triplicate water samples were collected from six major Ravi drains of Lahore i.e. Chota Ravi Drain, Gulshan-e-Ravi Drain, Saggian Drain, Hudiara Drain and Kharak Drain and transported to laboratory and stored at 4°C. The samples were collected in sterilized bottles (1.5 L) before the monsoon through grab sampling method, following standard sampling protocols. They were collected in such a way that no bubbles formed from the flowing water in the drains from any collection point. The bottles were sealed; labeled by the name of the site, date and time. All of the samples were preserved in an icebox at 4°C immediately after collection.

Selection of Insecticides/ Pesticides for analysis: Based on the information gathered by preliminary reconnaissance and interview based survey with the farmers, the insecticides/ pesticides selected for study include Cypermethrin, Deltomethrin, Bifenthrin and Chloropyrophos.

Analytical grade chemicals used in experiment are methanol, 25% aqueous ammonia, chloroform, n-hexane, acetone, 10% ammonia, dichoromethane, tetra-hydro-furan, cyclo-hexane, ether, benzene and distilled water.

Sample Preparation: 300 ml sample was mixed with 50 ml ethyl acetate and 50 ml dichloromethane. The sample was extracted twice with same solvents. The mixture was allowed to stand for 30 minutes in a separating funnel. Organic layer was collected, followed by the addition of anhydrous sodium sulfate. The mixture was then filtered after anhydrous sodium sulfate settled at the bottom. The solvents were evaporated in rotary evaporator and residues were dissolved in 5 mL ethyl acetate. The sample was stored in vial for HPLC and TLC analysis.

Analytical Technique Used

Thin Layer Chromatography: Thin layer chromatography was employed for checking and screening of insecticides and pesticides in samples against standards. Small volume of prepared samples and standards were applied to silica pre-coated TLC plates. TLC plates were developed in solvent systems described in Table 1. The plates were air dried for 30 minutes and observed under UV-light at 254 nm wavelength.

Table 1: Different Solvent Systems Tested for Insecticides and Pesticides

Sr No.	Solvent System	Ratio (ml)
1	Methanol: 25% Aqueous Ammonia	100: 15
2	Chloroform: Methanol	90: 10
3	n-Hexane: Acetone	5: 5
4	Methanol: 10% Ammonia	95: 5
5	Methanol: Dichloromethane: 10% Ammonia	5: 31: 4
6	Tetra-hydro-furan: n-Hexane	4: 6
7	Ethyle Acetate: Chloro-Hexane	1: 1
8	Ether	10
9	Dichloromethane	10
10	Ethyle Acetate	10
11	Benzene	10
12	Ether: Ethyle Acetate	8: 2
13	Ether: Ethyle Acetate: Methanol	8: 1: 1
14	Methanol	10
15	Ethyle Acetate: Methanol	5: 5
16	DCM: Methanol	5: 5
17	Methanol: Water	6: 4
18	n-Hexane	10
19	Methanol: 10% Ammonia	9: 1
20	Distilled water: Ammonia	9: 1
21	DCM: Methanol: 10% Ammonia	7: 2: 1

High Performance Liquid Chromatography: For further qualitative and quantitative determination of insecticides and pesticides HPLC was applied for all the prepared samples.

Apparatus for Analytical Analysis: The HPLC system having Agilene 1260 Quaternary Pump Gradient System with ODS 18 Column and DAD Diode detector was used for study.

Mobile Phases/ HPLC Conditions: Mobile Phase was HPLC grade acetonitrile and water in a ratio of 30:70. Both of them were filtered through cellulose filters (0.45 µm). All other parameters were optimized for determination of pesticides in study samples.

The run time was 45 min, flow rate was optimized as 1 ml/min, column temperature was kept at 25°C, injection volume was maintained at 10ìl and UV detection was done at maximum wavelength 245 nm and reference wavelength 360nm. The pH was adjusted to 4.5 with phosphoric acid. Before analytical analysis all water samples and standards were filtered through 0.45 ìm cellulose filter, acidified with HCl to pH < 2 and refrigerated at 4°C until analysis before being manual injection.

Method Development and Quality Control: A gradient easy and quite sensitive HPLC-UV method was developed and validated to determine chlorinated byproducts. To achieve method development, optimization studies were performed on each HPLC parameter like solvent ratio, pH, temperature of column, sample and injection volume, flow rate, wavelength and post time etc. System sensitivity, linearity and peak area reproducibility were also evaluated. For precise optimization, only one parameter was changed once keeping all other parameters constant. In order to access the precision and accuracy of the method European commission guidelines were followed and each water sample was analyzed in triplicate. For accuracy measurement the actual spiked value of samples were compared with the samples with known concentration of respective pesticides. Calibration experiments were tested for linearity, accuracy and precision. Limit of detection and quantification were considered as "an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantifieded as an exact value" and "an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy". LOD (3:1) and LOQ (10:1) were calculated as signal to noise ratio. In current study LOD was 0.01 while LOQ was 0.03 mg/L respectively.

Qualitative and Quantitative Analysis: Suspected pesticides were identified based on the retention times of the pure analytical standards.

For accurate quantification the formula formulated by chromo academy was used.

Response factor = Peak area of standard /Standard Amount Used in 1ml of Solvent

Amount of standard in sample = Peak area (sample peak)/ Response factor of selected byproduct.

RESULTS AND DISCUSSION

Focus of this study was to detect the presence of pesticides and insecticides in six drains of Lahore. For this purpose interview based survey was conducted in the study area and it was found that Chloropyrophos and Cypermethrin were the common pesticides, while Deltomethrin and Bifenthrin were common insecticide brands used in surrounding areas of these drains. For the purpose of analysis water samples were subjected, first, to TLC for each sample against standards. Previous studies also supported the use of TLC as rapid and inexpensive densitometric method for detection and determination of such persistent compounds in water [9,10]. Different combinations of solvents on silica gel TLC plates were tested for optimum identification of the investigated standard pesticides and insecticides. Ethyl Acetate; n-hexane and Methanol as a mobile phase in a saturated chamber proved to be most appropriate for identification of investigated pesticides and insecticides (Table 2). It was observed and is supported by studies that shorter development distance of the TLC plate lead to overlapping of the pesticides while development to longer distance gave good resolution results [9,10].

Among other chromatographic methods, HPLC was selected for detailed qualitative as well as quantitative analysis of pesticides and insecticides in water samples as HPLC is considered to be one of the valid analytical procedure for detection of such agrochemicals and their metabolites in the environmental samples [11]. Solvent system used in HPLC analysis was water: acetonitrile (1:1) as supported by literature for the present analysis. Standard procedures, with some modifications, were used for preparation of samples to run at HPLC. HPLC spectrum were interpreted on the basis of similarity in retention time

Table 2: Results of pesticides and insecticides detection via TLC

			Insecticides/ Pesticides detected	
Sr No.	Solvent System	Ration (ml)	Yes	No
1	Methanol: 25% Aqueous Ammonia	100: 15		P
2	Chloroform: Methanol	90: 10		P
3	n-Hexane: Acetone	5: 5		P
4	Methanol: 10% Ammonia	95: 5		P
5	Methanol: Dichloromethane: 10% Ammonia	5: 31: 4		P
6	Tetra-hydro-furan: n-Hexane	4: 6		P
7	Ethyle Acetate: Chloro-Hexane	1: 1		P
8	Ether	10		P
9	Dichloromethane	10		P
10	Ethyle Acetate	10	P	
11	Benzene	10		P
12	Ether: Ethyle Acetate	8: 2		P
13	Ether: Ethyle Acetate: Methanol	8: 1: 1		P
14	Methanol	10		P
15	Ethyle Acetate: Methanol	5: 5		P
16	DCM: Methanol	5: 5		P
17	Methanol: Water	6: 4		P
18	n-Hexane	10	P	
19	Methanol: 10% Ammonia	9: 1		P
20	Distilled water: Ammonia	9: 1		P
21	DCM: Methanol: 10% Ammonia	7: 2: 1		P
22	Methanol	10	P	

Table 3: Results of quantification of pesticides in different drains

		Insecticide (mg/l)		Pesticides (mg/l)	
Sr No.	Drain	Deltomethrin	Bifenthrin	Crypermethrin	Chorophyrophos
1	Chota Ravi	nd	nd	Nd	Nd
2	Gulshan-e-Ravi	nd	nd	Nd	Nd
3	Saggian	nd	nd	Nd	Nd
4	Hudiara	0.91	nd	7.17	441.02
5	Mian Mir	1.75	nd	6.23	383.3
6	Kharak	2.05	nd	0.47	29.04

nd = Not Detected

and peak area among samples and standards. By HPLC analysis Deltomethrin (insecticide), Cypermethrin and Chorophyrophos (Pesticides) were detected in Mian Mir, Hudiara and Kharak drain. Deltomethrin was present in concentrations of 0.91, 1.75 and 2.05 mg/L, respectively. On the other hand, two pesticides were reported in Hudaira, Mian Mir and Kharak drains; Chlorophyrosphos were reported in the concentration of 441.02, 383.3 and 29.04 mg/L in Hudaira, Mian Mir and Kharak drains respectively. Whereas Crypermethrin were detected in concentration of 7.17, 6.23 and 0.47 mg/L in Hudaira, Mian Mir and Kharak drains respectively (Table 3).

During the field survey for sampling, it was found that the extensive use of Deltomethrin, Chlorophyrosphos and Crypermethrin is common practice among farmers of surrounding areas to kill insects and pests. These compounds enter in drains in the form of irrigation or rain water run-off. The water of these drains is everyday in nearby fields to overcome water shortages. Due to this continuous cycle of application these compounds may be up taken by soil, vegetables, fruits and crops grown in fields. As demonstrated the pesticides become part of food chain causing adverse health impacts on consumers [9,12,13]. In developing countries like Pakistan, no significant efforts are made for the treatment of wastewater of drains which results in transfer of these toxic compounds in environment [14]. The detection of the persistent organic pesticides and insecticides in drain water which are already known to have adverse health effects, like retardation of central

nervous system of humans and disruption of enzyme activity, is alarming as this water is again used for agricultural purposes and enters the food chain posing impacts.

CONCLUSION

The use of excessive pesticides and insecticides disturbs the ecological system by degrading the quality of soil and reducing its nutrients through leaching into the soil, surface run-off or ground water contamination. Out of six drains pesticide Crypermethrin and Chorophyrophos while insecticide Deltomethrin have been detected and quantified in water samples of three drains. Chromatographic techniques of TLC and HPLC have been successfully employed for determination of selected pesticides and insecticides in this study. The pesticides which are detected in three drains are becoming a part of our food chain as during the dry season the major sources of irrigation are these drains. Throughout the year, the farmers use these drains to irrigate their crops thus appropriate control measures and regular monitoring to assess the amount of pesticides and insecticides should be conducted.

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