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Detection and Identification of multiresidual Pesticides in Water of Hudiara Drain and Its Adjoining Areas

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Key words: Pesticides, detection, retention times, drinking water, residual, abundance.

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Abstract

Pesticides play a vital role in green revolution by boosting the quality and quantity crops. The present study was designed for the detection and identification of multiresidual pesticides in water. The sources of environmental water samples were surface water and drinking water taken from the adjoining areas of Hudiara drain. An analytical method was produced and optimized for the detection and quantification of selected OPPs based on solid-phase extraction and high-performance liquid chromatography, coupled with a diode array detector (SPE–HPLC–DAD). The chromatographic separation was done using C18 column. Different organophosphorus pesticides (OPPs) were determined having specific peak areas, peak numbers, and their retention times along with their chemical structures. Each subsequent pesticide shows a unique behavior in three different solvents i.e., methanol, ethanol and acetonitrile in the water samples for the optimization of micro-pesticides in drinking water. The presence of large amount of pesticides in the sampling areas is due to the manufacturing industry for pesticides and agriculture farms in the adjoining areas. The measures should be taken for monitoring and controlling the use of pesticides according to permissible limits.

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Introduction

A vast variety of organic and inorganic xenobiotics are annually flushed in to the water, soil and air resources of the environment. These substances have the capability of harming man or any other living organisms, which is dependent on the environment. According to Appannagari 2017, the environmental disasters are caused due to environment or ecological changes due to developmental process of the 'technologic and economical man" of the current century. The environmental disasters are arising due to environmental deterioration caused by various forms of pollution, depletion of resources because of increasing exploitation, dependence on energy consuming and ecologically damaging technologies, the loss of habitats because of industrial, urban and agricultural development, reduction and loss of ecological species due to exacerbated use of toxic/harmful pesticides/herbicides and loss of several plant species due to monoculture removal of habitats through deforestation has now become a global concern (Tankiewicz and Biziuk, 2018).

Human life is being adversely affected by anthropogenic environmental degradation that there has been a striking growth of interest within the last decade in the quality of the environment, the depletion of resources and disruption of the earth's natural ecosystems.

A vast number of synthetic organic compounds have been produced from industrial, domestic, or agricultural practices. Some portion of the organic compounds used for industrial or domestic purposes will enter wastewater as part of the influent. Unless specifically removed by efficient treatment processes, they may persist as part of the effluent and be released into receiving waters as trace pollutants (Daughton, 2003; Heberer, 2002; Lishman *et al.*, 2006). Some fraction of the organic compounds used for agricultural purposes will runoff into a surface water body, while another fraction of the compounds will infiltrate and reach the groundwater system. The receiving waters for trace pollutants may be a direct source of drinking water or indirectly reach a water supply as recharge water. The term trace pollutant indicates low concentrations of an environmental contaminant normally in the nanogram (ng) or microgram per liter range. Trace pollutants receiving the most attention by researchers and regulators generally belong to one of the four broad groups 1) industrials, 2) pesticides, or 3) pharmaceuticals and personal care products (PPCPs) (Murray *et al.*, 2010).

Developments in agricultural productions have resulted in broad application of the chemical fungicides (Firoz *et al.*, 2016). Residual pesticides have easy access to the water, soil, atmosphere and biosphere, but some pesticides are persisting in both groundwater and surface water (Caldas *et al.*, 2013; Sanchez-Gonzalez *et al.*, 2013). They cannot still be altogether removed by the process of present drinking water treatment techniques (sediment/filter/disinfection/coagulation), so the threats due to these pesticides in drinking water are also rising (Yu, 2015). With the growing plant diseases and to meet the need of food for growing population, large amount of fungicides and pesticides are used to reduce the disease burden and enhance the crop yield (Soliman *et,al.*,2013).

The objective of this work was Evaluation of preconcentration techniques of trace pesticides through Solid Phase Extraction (SPE) for the efficient detection of trace pesticides in the drinking water by HPLC.

Materials and methods

The present study was conducted for the detection and identification of multi residual pesticides in water. The main sources of water samples were surface water and drinking water. According to this experiment and for necessary analysis, water samples were processed. The sample was collected from the adjoining zone of hudiara drain near DHA. They were preserved and transported according to Wang and Li, 2015. The preconcentration was followed by liquid–liquid extraction and analysis was done with reverse phase- High performance liquid chromatography coupled with photodiode array detector.

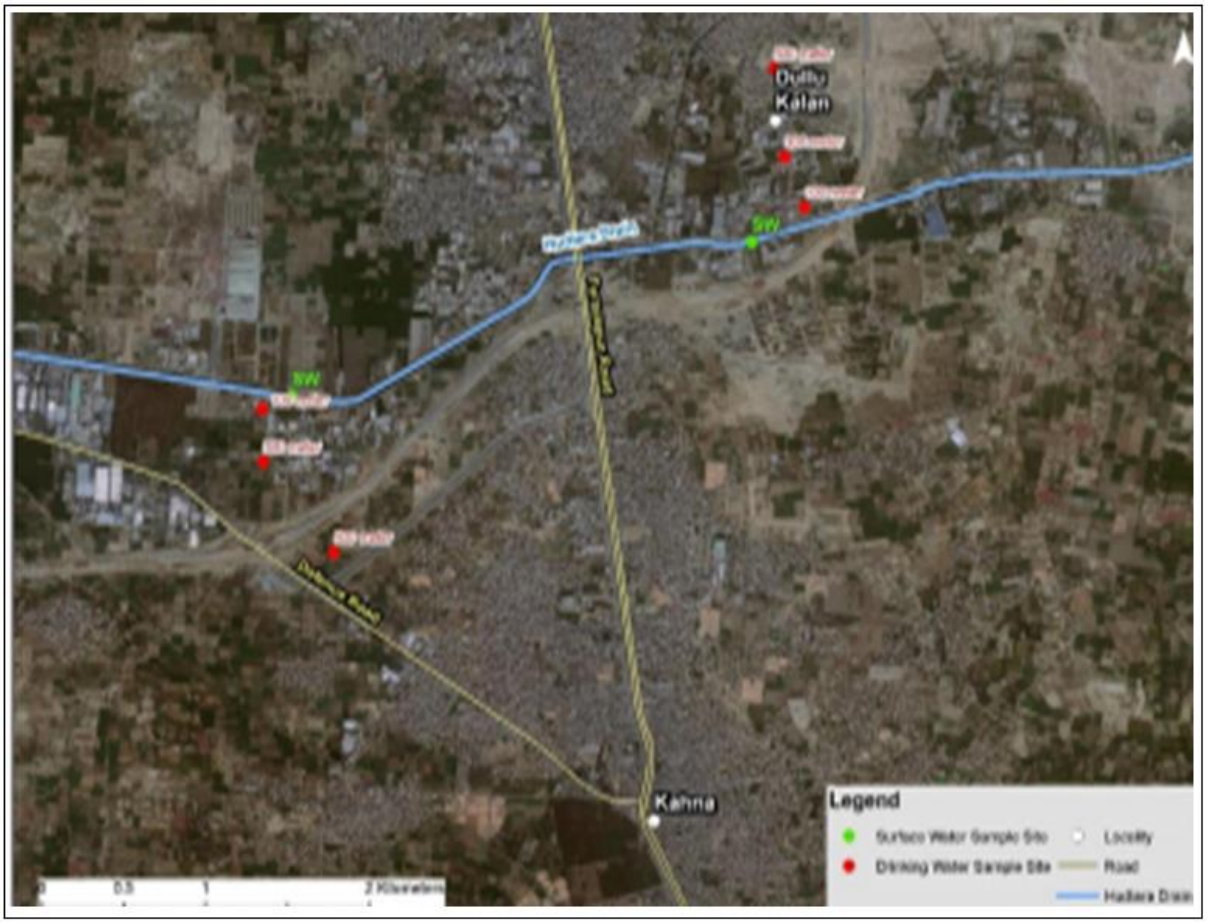

Fig. 1. Map of the study area and subsequent sampling location.

Sampling location

Water samples were collected from near Hudiara drain and its adjoining areas near Lahore. A total of ten samples of water were taken from the Dullu Kalan area near Gajju Matta. (Figure.1) Simple random sampling was done to obtain the water samples from the selected sites of Hudiara drain and consequently also from the drinking water (Table 1). The sample is the representative points of the source of supply.

Collection of samples

For the purpose of surface water sample collection, the amber bottle used, was gripped firmly and the neck of the bottle plunged downwards to a depth of about 0.5m. The bottle was twisted until the neck points upward and mouth is directed towards the current. The taps in the area of test was chosen which is most frequently used for taking the samples. Any exterior fittings were detached such as filters and remove any contaminants (e.g. grease, slime, sediment build-up) around the spout with a clean cloth. Tap hygiene is particularly important with microbiological testing. Before taking the sample, Tap outlets which are suspected of the contamination are disinfected firstly. Disinfect by washing the outside of the tap and as much of the inside as possible with a 0.1% sodium hypochlorite solution AS/NZS.

Preservation and storage

The preservative added was sodium thiosulphate for pesticides to the empty sample containers for the preservation of samples. For pesticide samples, the water was collected to fill the container to near full capacity. The bottle was wrapped with the top ensuring that the top for pesticides contains an inner Teflon liner seal. These water samples were carefully filled to the brim to avoid trapping air. After filling up the bottles, they were sealed with Teflon lined screw caps and these bottles were then kept in ice for transportation to the laboratory before 24 hrs where they were stored at 4 degrees Celsius for extraction and analysis.

Transportation

The properly labeled bottles were placed in an ice box at 4°C (using frozen briquettes) or other suitable container for transportation to the laboratory.

Physical and chemical parameters

The physiochemical parameters such as pH, temperature, electrical conductivity and total dissolved solid were determined soon in the lab. They were observed by Ino lab pH meter (MARTINI, Mi-160), Ino Lab (Cond Level 1) electrical conductivity meter, Ino Lab Cond 730-Fagerberg, following the standard procedures.

Chemicals and reagents

Ethanol (>99%), methanol (>99%), acetonitrile (>99%), were of analytical grade and taken from Merck.

Equipments

Beakers 100ml, conical flask 100ml, measuring cylinder 100ml, funnel, plastic vials, stirrer, aluminum foil, water bath (memmert), Hot plate (IRMECO MSC Digital), Laminar flow (ARIASReinraum Techik GmbH, CJ- 1FD), Fume Hood (DEH 10), pH meter (MARTINI, Mi-160), InoLab (Cond Level 1) electrical conductivity meter,centrifuge machine (Sigma 2-6), Ultrasonic bath sonicator (Elma E 30-H), inoLab Cond 730-Fagerberg and HPLC (waters 2996 photodiode Array Detector: waters 600 Controllers Pump).

Liquid –liquid extraction procedure

Sample preparation

The 100ml of each sample were taken in a measuring cylinder. The funnel was placed on the top of measuring cylinder and then exact amount of samples were poured in beakers to pre-concentrate the samples to 5ml from 100 ml of that surface and drinking water samples. The beakers were placed on hot plate at temperature 340℃. The heated samples were pre-concentrated for some time to meet the exact amount of samples for our experiment on hot plate. When the samples became pre-concentrated to 5ml, they were cooled and the Volume was noted.

Addition of the extraction solvents

Three different extraction solvents of pesticidal polarity were considered to assess extraction procedure. They are as follows: • Ethanol (100%) • Methanol (100%) • Acetonitrile (ACN) (100%) Ethanol was detached to make the ratio of 30: 5 levels of beakers having concentrated samples. The samples were moved into the conical flasks of 100 ml each and covered by aluminum foils to evade evaporation after cooling. The subsequent samples then were fitted in the water bath shaker overnight. The main purpose of using a water bath shaker is to shake the sample steadily and mix them while sustaining a constant temperature. Flasks were engaged for sonification for the agitation of the samples with the organic solvent for almost 24 hours at temperature 28℃ after the shaking. After that samples were poured in the vials (plastic) to level of 12 µl and then centrifuge the samples. The 10 each subsequent sample were involved with the ethanol, 32 methanol, and acetonitrile for the subsequent procedure. After that the exact protocol of high performance liquid chromatography (HPLC) was used for the detection of optimization of the micro-pesticides in drinking water.

Operating procedure of HPLC for method development / chromatographic conditions:

The analysis of pesticides was carried out using reverse phase high performance liquid chromatography coupled with (waters 2996 photodiode Array Detector: waters 600 Controllers Pump) photodiode array detector. The chromatographic separation was carried out using C18 column with dimensions of 250 4.6 mm and pore size of 5 mm. The mobile phase contained acetonitrile (A) and HPLC water (B). The mobile phase was filtered using 0.22mm membrane and degassed for 30 min by sonication. The gradient elution started with being 45% and 55% B, continued at 30% at 5.00 min., 20% at 7.50 mins, gradually 25% at 9.00 min., followed by 35% at 14.01 min. and stopped at 18.01 min. The flow rate was maintained at 1.00 mL/ min, 12mL injection volume, 38 °C column temperature and the maximum pressure limit of 15,000 psi (Zhang

et al., 2018).

Statistical analysis

Results obtained were analyzed statistically for water samples and pesticide metabolites. The analysis was conducted on Graph Pad Prism vs 6.1. T-tests and ANOVA were performed as per different experiments and results were deduced out of it.

Results and discussion

The present study was conducted for the purpose of detection and identification of pesticides residues in drinking water in the selected suburban area.

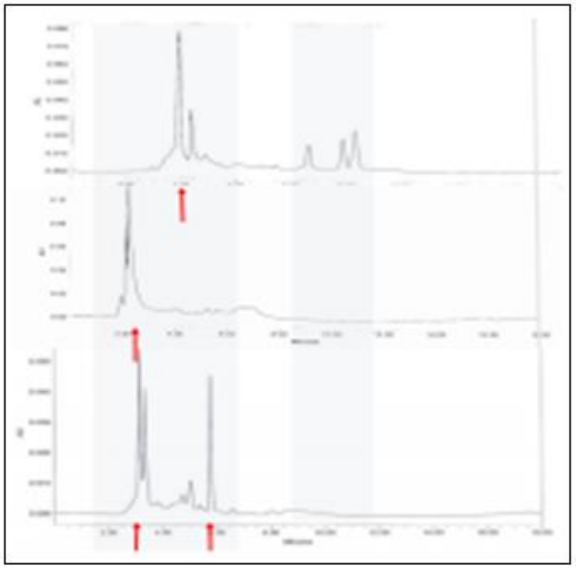

Fig. 2. Comparison of peaks from S1M, S1E, S1ACN.

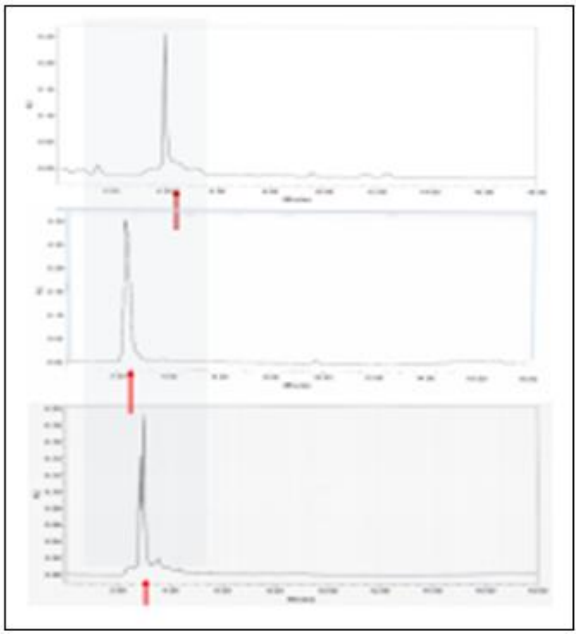

Fig. 3. Comparison of peaks from S2M, S2E, S2ACN

The preconcentration method of liquid–liquid extraction was performed from analysis by reverse phase- High performance liquid chromatography coupled with photodiode array detector for the detection of trace multiresidual pesticides (Yadav *et al.*, 2015).

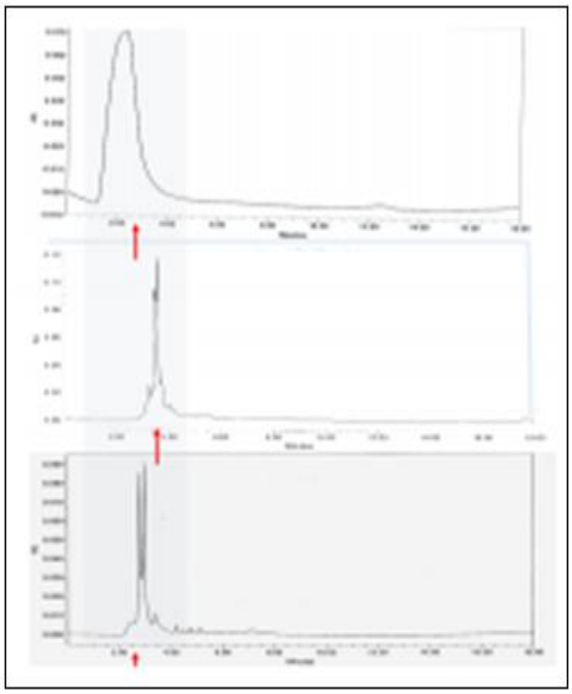

Fig. 4. Comparison of peaks from S3M, S3E, S3ACN.

The chromatographic separation was done using C18 column with dimensions of 250 4.6 mm and pore size of 5 mm. The mobile phase used consisted of acetonitrile (A) and HPLC water (B).

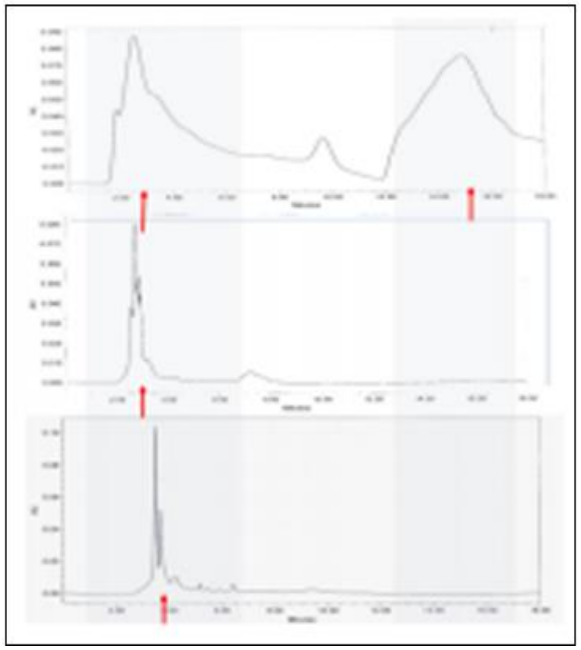

Fig. 5. Comparison of peaks from S4M, S4E, S4ACN.

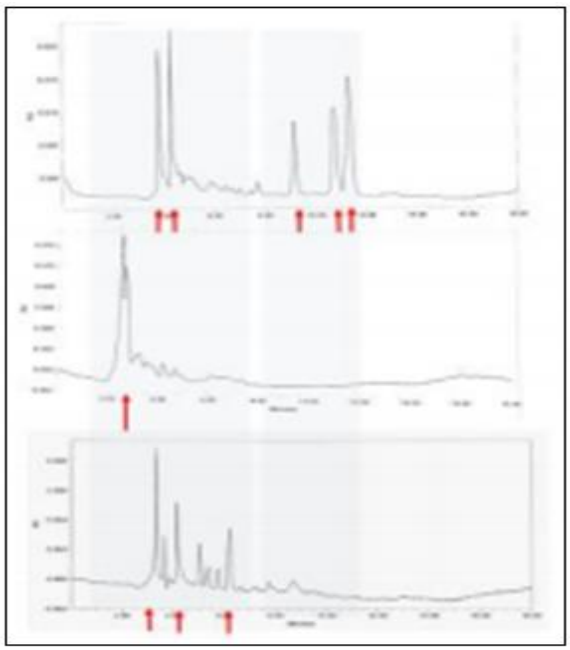

Fig. 6. Comparison of peaks from S5M, S5E, S5ACN.

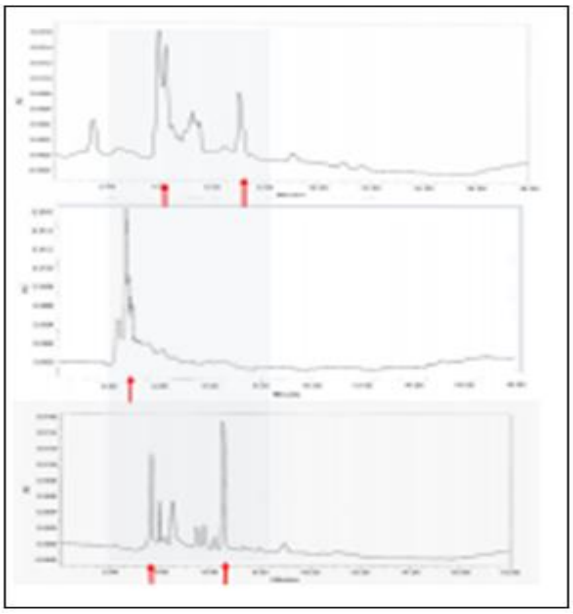

Fig. 7. Comparison of peaks from S6M, S6E, S6ACN.

The results of expected pesticides extracted from methanol include the methyl parathion, malathion, Beta Hexachloropyclohexane from the sample S1M. Pesticide identified in sample S2M was malathion only.

The S3M points toward the expected presence of BetaHexachloropyclohexane. S4M indicates the presence of BetaHexachloropyclohexane and Ethoprophorus. The solution with most abundance of peaks was S5M. It has the peaks of Methyl parathion, malathion, diazinon, Dichlorvos, permethrin.

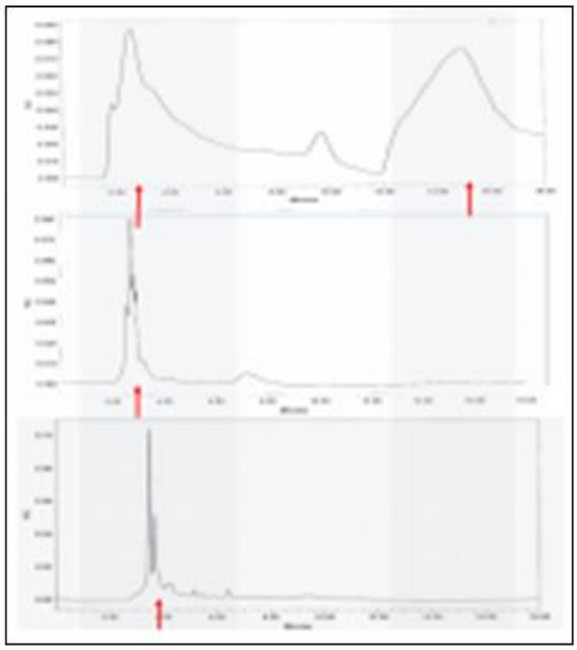

Fig. 8. Comparison of peaks from S7M, S7E, S7ACN.

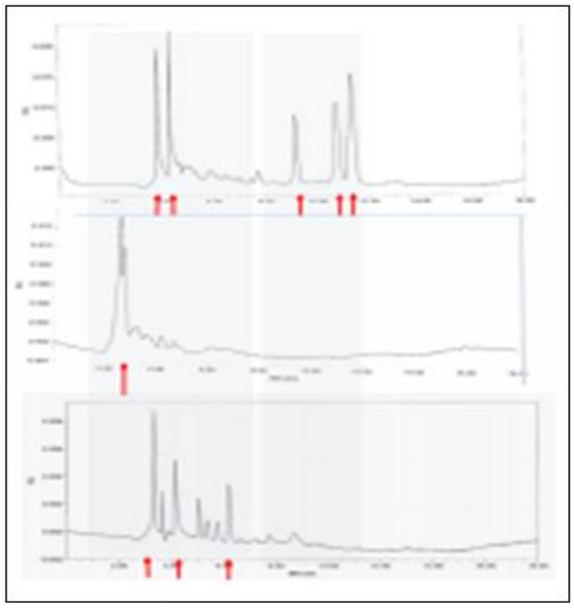

Fig. 9. Comparison of peaks from S8M, S8E, S8ACN.

This processed sample contained the abundance of pesticides. Simultaneously S6M had three peaks of methyl parathion, malathion, along with Endosulphan β. The sample S7M only contained a single peak of 34 cypermethrin. The Sample S8M have two peaks of methyl parathion, malathion but only S10M had peak of Endosulphan state Organochlorine and nitrogen containing pesticides are banned in Pakistan but there is no monitoring to make sure.

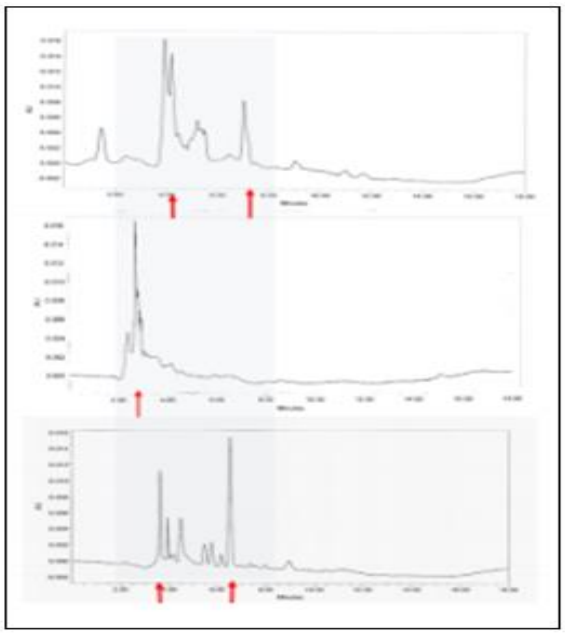

Fig. 10. Comparison of peaks from S10M, S10E, S10ACN.

That is the reason of presence of certain concentration of pesticides in river water. DDT, DDE, endosulfan and carbofuran can be found in these water bodies (Affum *et al.*, 2018). The most abundant expected pesticide extracted from methanol was methyl parathion, malathion with four and five peaks respectively (Fig. 11). Organophosphorus pesticides (OPPs) which are the alternative to organochlorine pesticides (OCPs) contain organophosphate compounds (Wee *et al.*, 2016). However, recent studies show that they can be of high toxicity and can act as a carcinogen. Different OPPs have specific peak areas, peak numbers and retention time with their chemical structures. The estimation of pesticide abundance and their peaks could be analyzed by HPLC in drinking or surface water (Sanchez-Santed *et al.*, 2016).

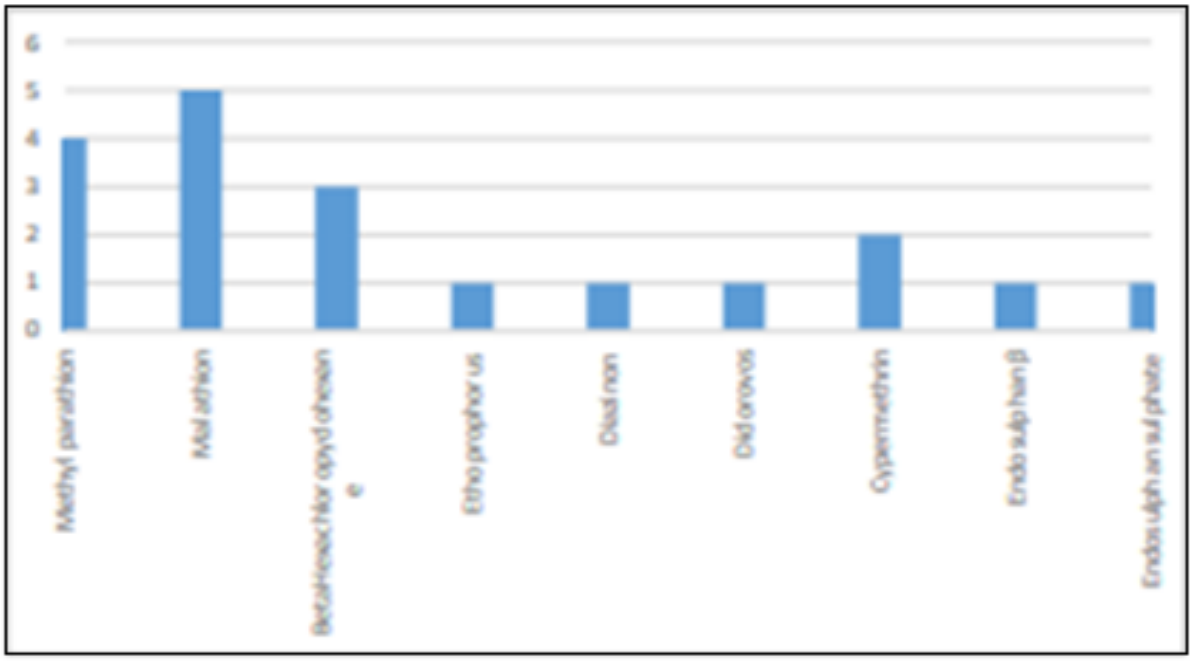

Fig. 11. Abundance of expected pesticide peaks extracted from methane.

As shown in the (Fig. 12), the most abundant peaks were of n C12 origin followed by β-HCH. The expected peaks of pesticides extracted from the acetonitrile were the heptachlor, Diclorovos Chloropyrifos-methyl from the sample S1ACN. Sample S2ACN and S3ACN had the two peaks of Diclorovos and Imidacloprid respectively (Fig. 13). Peak areas also relate to a conclusion that the particular pesticides cover more areas then others. It corresponds to their abundance in a sample from a specific area. Compounds of Peak 5 and 6 had highest and higher peaks respectively then others. Peak 4 also had high abundance with respect to other peak areas (Fig. 14).

The results extracted from ethanol have different results, the pesticides with namely peak 3 and 7 had more area then other corresponding to the abundance

of compound in a sample. The peaks 2 and 9 did not disappoint either they followed in abundance after the peak 3 and 7 respectively. (Fig. 15) Ionic liquids like hydrophobic ionic liquids incorporating the imidazolium cation and hexafluorophosphate anion have higher density than water. They are more compatible with reversed-phase HPLC due to the non-harmfulness to column, compared with commonly used solvents. (Feng *et al.*,2020).

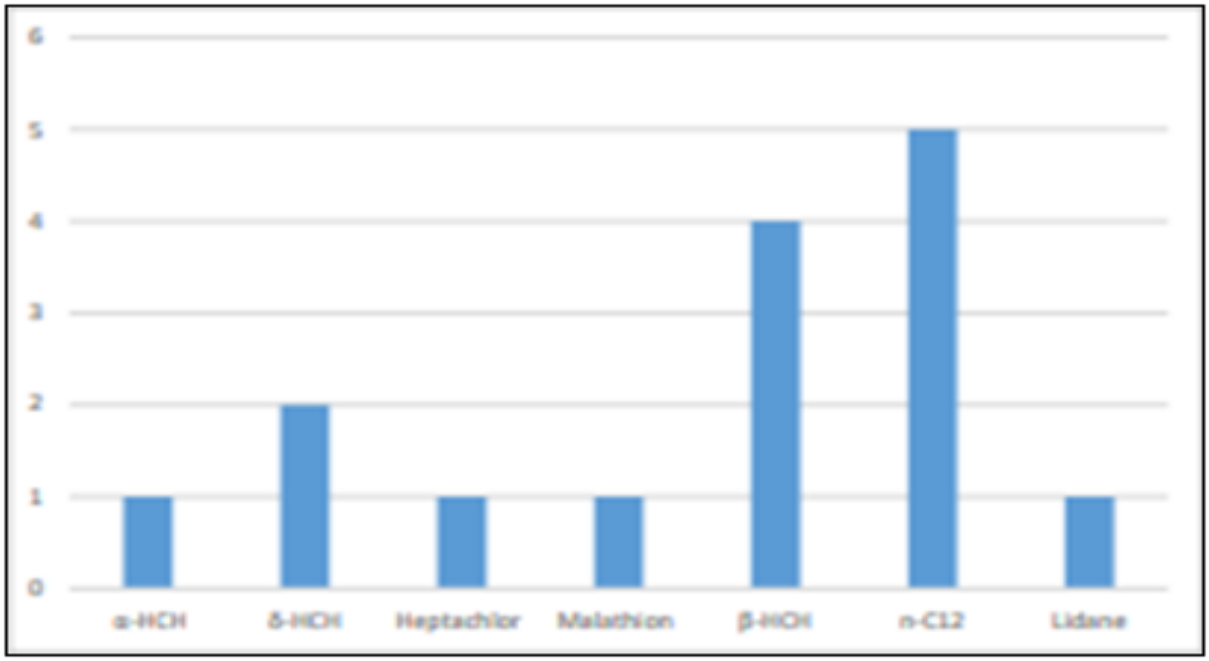

Fig. 12. Abundance of expected peaks extracted from ethanol.

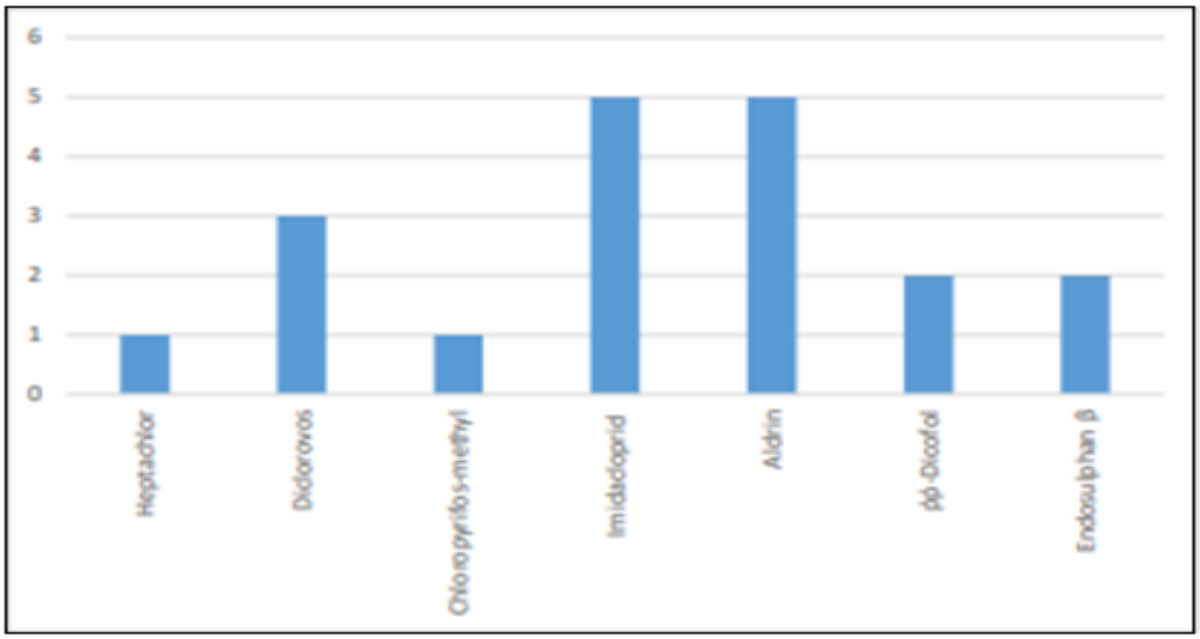

Fig. 13. Abundance of expected peaks extracted from acetonitrile.

Fig. 16 shows that the peaks 2, 4 have more peak areas following peak 8 corresponding to the abundance of pesticide in the particular area and sample. The results of the present study shows that the method is simple, fast, and uses environmentally friendly extraction solvent for the detection of the target pesticide residues in environmental water samples (Yang *et al.*,2021).

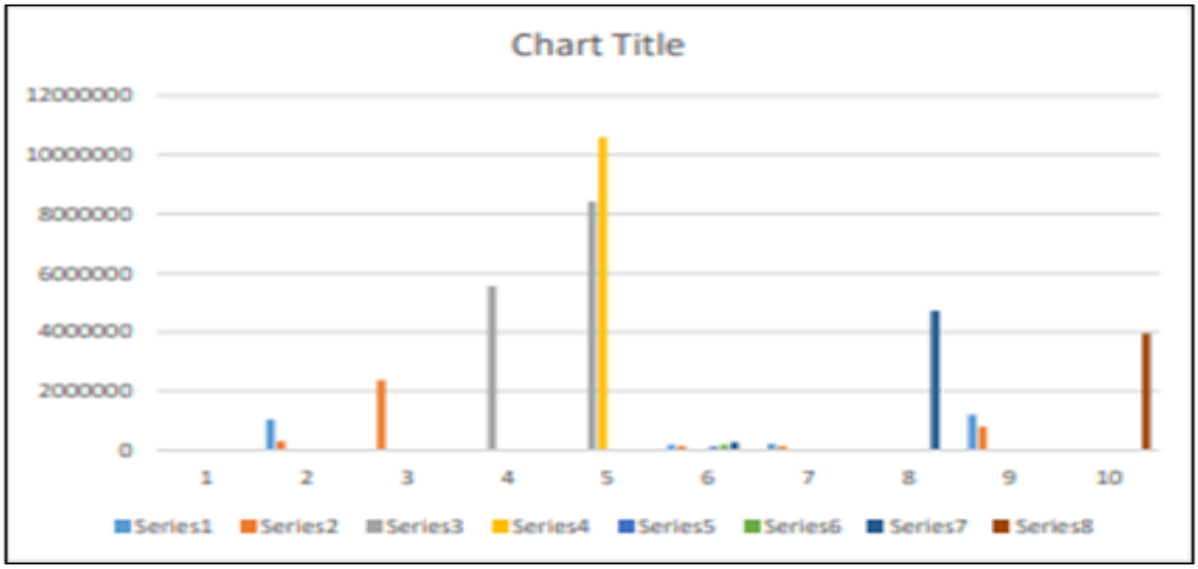

Fig. 14. Peak areas of expected pesticides extracted from methanol.

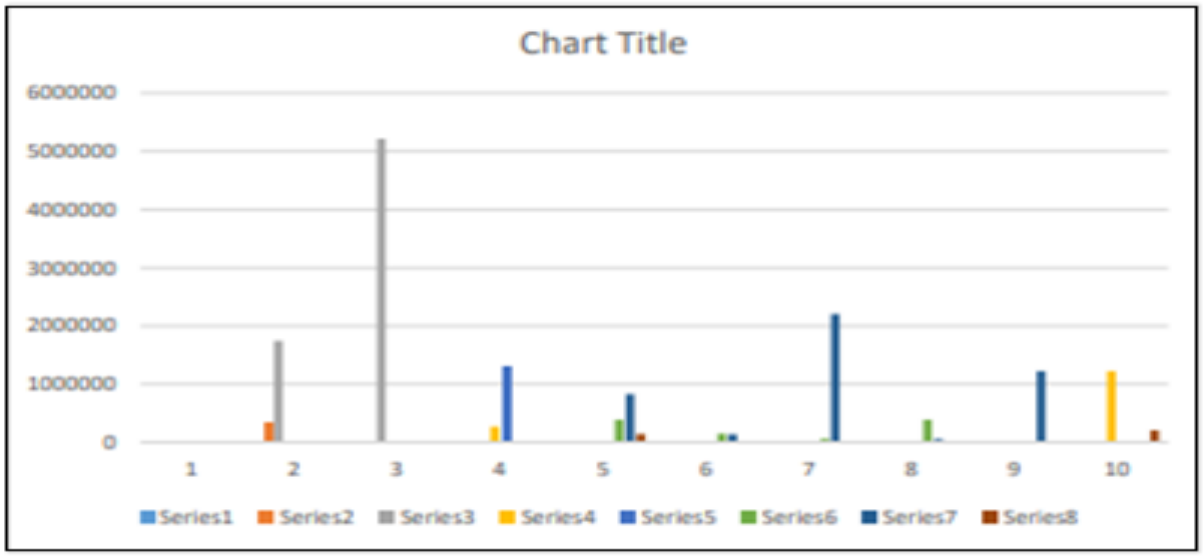

Fig. 15. Peak areas of expected pesticides extracted from ethanol.

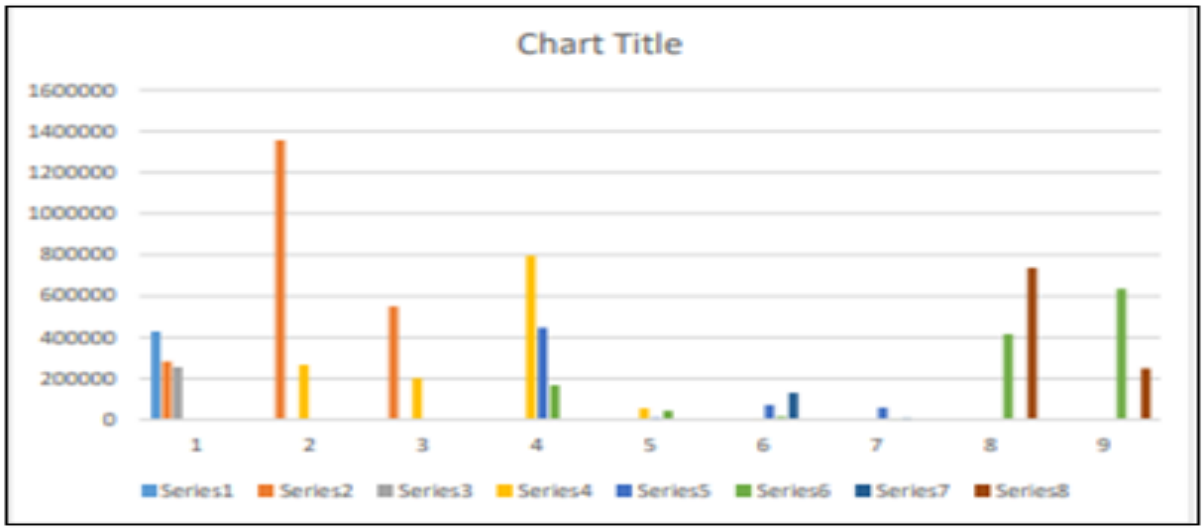

Fig. 16. Peak areas of expected pesticides extracted from acetonitrile.

Conclusion

The three extraction solvents were used to evaluate the preconcentration technique with liquid- liquid extraction by using reverse phase high performance liquid chromatography coupled with photodiode array detector. As the results show that methanol was the solvent which attained higher no of peaks up to 8. The peaks extracted from acetonitrile and ethanol were 6 and 7 individually. This corresponds to the attraction of different pesticides with different extraction solvents in high performance liquid chromatography. The expected peaks of pesticides were related on the basis of their survival time. The large amount of pesticides in the sampling water areas is due to the presence of agricultural farm and manufacturing industries in the corresponding areas. There should be appropriate protective measures by authorities to control the use of pesticides and to maintain their limits.

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