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HPLC Determination and Comparative Analysis of Persistent Organic Compounds in Different Environmental Matrices

NIKHAT KHAN[1], MAHAM ALI[1], GHAZALA YAQUB[1,*] and ZUBI SADIQ[2]

[1]Department of Environmental Sciences, Kinnaird College for Women, Lahore-54000, Pakistan
[2]Department of Chemistry, Lahore College for Women University, Lahore-54000, Pakistan

*Corresponding author: Tel: +92 331 4063466; E-mail: ghazala_yaqub@yahoo.com

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The application of easy, low-cost and effective sample preparation approach for the determination of different categories of persistent organic compounds, fruit, vegetable, soil and water samples was conducted. After extraction of environmental matrices, targeted persistent residues of lufenron, imidacloprid, dimethomorph, paraquat, glyphosate, bifenthrin and difenoconazole were determined by the method validation of high performance liquid chromatography in the collected samples from the agricultural areas of Lahore and Rahim Yaar Khan, Pakistan. All fruits and vegetables were found to be contaminated with persistent organic compounds with the values exceeding maximum residue limits (MRLs) of Codex Alimentarius Commission. Present study is a contribution in scientific research that gives the comparative analysis of persistent organic compounds under various environmental conditions.

Keywords: Persistent organic compounds, High performance liquid chromatography, Maximum residue limits.

INTRODUCTION

Persistent organic pollutants (POPs) exist for long time, migrating in air, water, soil and sediments, accruing to levels that could harm wildlife and human health [1]. These are natural or anthropogenic origin with a particular combination of physical and chemical properties *i.e.* once released in the environment, they remain undamaged for very long period and resist photolytic, chemical and biological degradation [2,3]. Because of their toxicity and tendency to accumulate in food chains, persistent organic pollutants are a matter of concern lately [4]. The persistence of contaminants in the Arctic from distant sources came when pesticides were found in polar bear fat tissue, shows reality of persistent organic pollutants on wildlife and human health researches have started to explore for evidence of airborne persistent organic pollutants in cold ecosystems and mountainous environment [5]. In August 1989, Canada presented a report on hazardous persistent organic chemicals [6].

Chromatography appears to be the most promising technique for the monitoring of persistent organic compounds (pesticide) residue in environmental matrices due to its high component separation power. Several chromatographic techniques used for reporting the concentration of persistent organic compounds in different environmental matrices are shown in Table-1. High performance liquid chromatography is used as the method of choice for the detection of persistent organic compounds in fruits, vegetables, soil and water samples [7]. The research undertaken here reports a single HPLC assay that precisely analyze residual lufenron, imidacloprid, dimethomorph, paraquat, glyphosate, bifenthrin and difenoconazol concentrations in vegetables, fruit, soil and water samples collected from agricultural areas of Lahore and Rahim Yaar Khan, Pakistan. The chemical properties of persistent organic compounds are shown in Table-2.

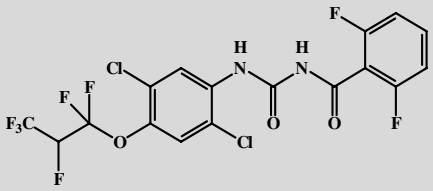
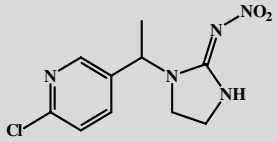
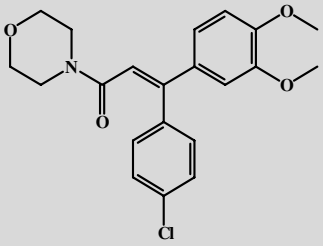
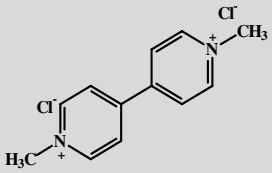
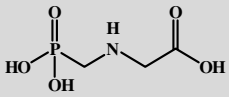
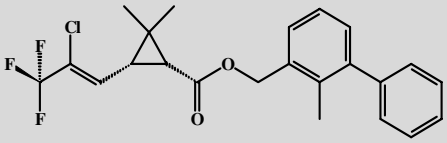
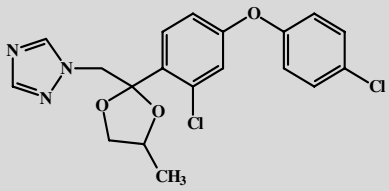
TABLE-1
PERSISTENT ORGANIC COMPOUNDS DETERMINATION BY VARIOUS CHROMATOGRAPHIC TECHNIQUES

Environmental matrix	Analyte	Method	Ref.
Fruits	Pesticides	GC-MS, HPTLC,	[8,9]
Vegetables	Pesticide	GC-MS, HPLC, GC-NDP/FID	[7,10]
Soil	Pesticide	GC-MS/ECD, HPLC-MS	[11,12]
Water	Pesticide	GC-MS-MS, HPLC ECD,	[13,14]

EXPERIMENTAL

Two fruit samples (shakri malta, mango), two vegetable samples (pumpkin, lemon), three soil samples (fruit soil, vegetable soil, vegetable + fruit soil) and four water samples (canal water, tube well water, still water in fields, drain water) were collected from the agricultural areas of Lahore and Rahim

TABLE-2
PERSISTENT ORGANIC COMPOUNDS CHEMICAL INFORMATION (EUROPEAN UNION PESTICIDE DATABASE)

Compounds	Chemical structure
Lufenron (Pesticide) Family: Benzoylurea	
Imidacloprid (Pesticide) Family: Neonicotinoid	
Dimethomorph (Fungicide) Family: Morpholine	
Paraquat (Herbicide) Family: Voilogen	
Glyphosate (Herbicide) Family: Glycine derivative	
Bifenthrin (Insecticide) Family: Pyrethroid	
Difenoconazole Fungicide Family: Chlorophenyl ether	

Yaar Khan, Pakistan. Samples were transported to laboratory at normal environmental conditions.

The analytical grade chemicals used in the experiment are, ethyl acetate, dichloromethane, sodium chloride, sodium sulfate and methanol.

Sample preparation

Vegetables and fruits samples preparation: Accurately 1 kg of vegetable and fruit samples was air dried and grinded into a miller to fine powder. Precisely 2 g of the powdered sample was transferred to a beaker followed by the addition of 75 mL distilled water, 2.5 g NaCl, 20 mL ethyl acetate and 20 mL dichloromethane. The mixture was stirred on a magnetic stirrer at 4 rpm for 15 min. Using a strainer the mixture was strained to remove the solid particles. The mixture was poured in a separating funnel and allowed to stand for 15-20 min. The organic layer (lower layer) was collected from the separating funnel. Aqueous layer was again extracted with 10 mL ethyl acetate and 10 mL dichloromethane.

Soil samples preparation: About 0.5 kg of soil samples were air dried. Accurately 5 g of the air dried sample was transferred to a beaker followed by the addition of 200 mL distilled water, 40 mL ethyl acetate and 40 mL dichloromethane. The mixture was stirred on a magnetic stirrer at 4 rpm for 15 min and poured in a separating funnel and allowed to stand for 15-20 min. The organic layer (lower layer) was collected from the separating funnel. Aqueous layer was again extracted with 20 mL ethyl acetate and 20 mL dichloromethane.

Water samples preparation: Precisely 200 mL of sample was transferred to a beaker followed by the addition of 200 mL distilled water, 40 mL ethyl acetate and 40 mL dichloromethane. The mixture was stirred on a magnetic stirrer at 4 rpm for 15 min and poured in a separating funnel and allowed to stand for 15-20 min. The organic layer (lower layer) was collected from the separating funnel. Aqueous layer was again extracted with 20 mL ethyl acetate and 20 mL dichloromethane.

Standard preparation: Accurately 1 g standard sample was transferred to a beaker followed by the addition of 30 mL distilled water, 0.75 g NaCl, 15 mL ethyl acetate and 15 mL dichloromethane. The mixture was stirred on a magnetic stirrer at 4 rpm for 15 min and poured in a separating funnel. It was allowed to stand for 15-20 min. The organic layer (lower layer) was collected from the separating funnel. Aqueous layer was again extracted with 7.5 mL ethyl acetate and 7.5 mL dichloromethane.

Clean-up: Sodium sulfate was added for sample and standard clean-up. The collected organic layer was transferred to beaker; 10-20 g Na_2SO_4 was added and kept for 20 min by intermittent stirring. The content was filtered using No. 42 Whatmann filter paper. The filtrate was evaporated in a rotary evaporator at 40 °C. The residues obtained were dissolved in 20 mL of methanol. Fig. 1 illustrate the sample preparation procedural flow chart.

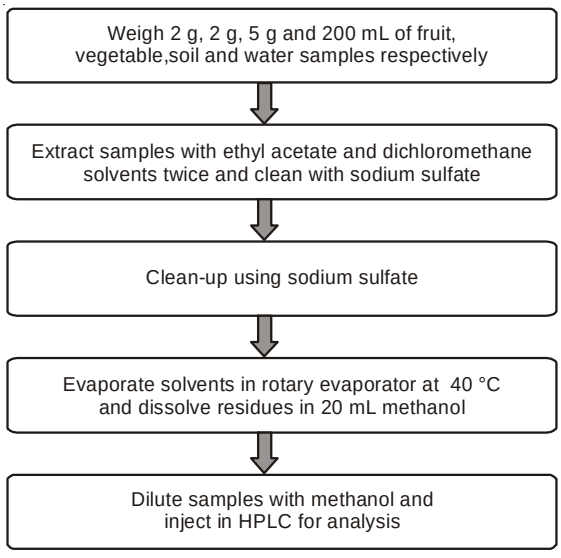


Fig. 1. Sample preparation procedural flow chart

Analytical technique: For analysis samples and standards were further diluted with methanol and injected in 5 µL volume in HPLC Agilent 1260 quaternary gradient system fitted with diode UV/visible detector set at 254 nm and C18 Licospher column. The mobile phase contained acetonitrile: water in the ratio of 40:60 having a flow rate of 1 mL/min at 25 °C column temperature.

RESULTS AND DISCUSSION

The key objective of this research was to create easy, low-cost and a successful method to determine persistent organic compounds in the fruits, vegetable, soil and water. Several solvent systems were tested for their effectiveness but ethyl acetate + dichloromethane solvent system was verified to be most proficient for pesticide HPLC analysis. The final cleaned up extract of some samples appeared to be light yellow or green. But with powerful selectivity of HPLC (Agilent 1260 fitted with C18 Licospher column) the extracted samples appeared to be clean and free of co-eluting impurities, indicating that the cleaned-up samples did not contribute any interference with target persistent organic compounds to be analyzed.

HPLC results were quantified by response factor method by using following formulas

$$\text{Response factor} = \frac{\text{Peak area}}{\text{Standard amount}}$$

Concentrations of analyte in each sample were calculated by:

$$\text{Amount of analyte} = \frac{\text{Peak area}}{\text{Response factor}}$$

By utilizing above illustrated formulae, the resultant concentration of persistent organic compounds obtained in vegetables (pumpkin, lemon), fruits (shakri malta, mango), soil (vegetable soil, fruit soil, vegetable + fruit soil) and water (canal water, tube well water, drain water, still water in fields) were calculated (Table-3).

Paraquat, a broad spectrum herbicide, belongs to bipyridylium family is also known as methyl viologen [15]. Surprisingly, only paraquat was detected in Lahore samples in concentrations of 6.25 mg/Kg, 5.06 mg/Kg, 40.39 mg/Kg, 12.07 mg/kg, 4.74 mg/Kg, 0.11 mg/L and 0.104 mg/L in shakri malta, mango, pumpkin, lemon, vegetable + fruit soil, canal water and tube well water respectively. The persistence of paraquat in soil is reported by several studies [16]. It strongly binds itself with the organic content of soil due to low vapor pressure and extremely high adsorbing coefficient (RED). The reason of high concentration of paraquat in vegetables and fruits is due to its ability of bio-accumulation (Hristov). It is transported to fruits and vegetables growing in contaminated soil and water coming in contact with the soil [17]. In many countries the use of paraquat products has been restricted and even banned due to its high toxicity [18].

Bifenthrin, an insecticide [19], belongs to pyrethroid group [20]. In Rahim Yaar Khan samples bifenhtrin was recorded in quantities of 131.15 mg/Kg, 210.1 mg/Kg, 325.29 mg/Kg, 273.53 mg/Kg, 1.47 mg/Kg, 1.13 mg/Kg, 3.44 mg/L, 3.56 mg/L and 0.03 mg/L in shakri malta, mango, pumpkin, lemon, vegetables soil, fruit soil, canal water, still water in fields and drain water respectively. The high concentration of bifenthrin in tested samples can be correlated with high persistence in environmental matrices with half-life of 7 days to 8 months [21].

Difenoconazole, a broad spectrum nitrogen containing fungicide, belongs to triazole group [22]. In Rahim Yaar Khan samples difenoconazole were reported in concentrations of 209.31 mg/kg, 333.26 mg/kg, 515.1 mg/kg, 433.16 mg/kg, 2.33 mg/kg, 1.8 mg/kg, 0.05 mg/L, 5.64 mg/L, 5.44 mg/L in shakri malta, mango, pumpkin, lemon, vegetables soil, fruit soil, canal water, still water in fields and drain water respectively. Several studies have reported the persistent nature of difenoconazole in soil [23]. Elevated concentration in vegetables and fruits samples is associated with the high persistence of difenoconazole in soil as is a medium of growth for vegetables and fruit [24].

Fruits and vegetable samples from Lahore and Rahim Yaar Khan were found infected with persistent organic compounds with quantities above maximum residue limits. Table-4 demonstrates the comparison of recorded quantities of pesticides in vegetable and fruit samples with maximum residue limits. For soil and water no standards have been established to compare with, but soil and water samples also show appreciably high quantities of pesticides. The presences of very high quantities of residual persistent organic compounds in all fruits, vegetables, soil and water samples reflects

TABLE-3
RESULTANT CONCENTRATION OF PERSISTENT ORGANIC COMPOUNDS IN SAMPLES

Sample	Sample name	Detected persistent organic compounds		
		Lahore	RahimYaar Khan	
		Paraquat concentration	Difenoconazole concentration	Bifenthrin concentration
Fruit	Shakri malta	9.265 mg/kg	209.31 mg/kg	132.15 mg/kg
	Mango	5.0645 mg/kg	333.26 mg/kg	210 mg/kg
Vegetable	Pumpkin	40.395 mg/kg	515.1 mg/kg	325.29 mg/kg
	Lemon	12.07 mg/kg	433.16 mg/kg	273.53 mg/kg
Soil	Vegetable soil	Not detected	2.33 mg/kg	1.476 mg/kg
	Fruit Soil	Not detected	1.8 mg/kg	1.138 mg/kg
	Vegetable + Fruit soil	4.745 mg/kg	Not detected	Not detected
Water	Canal water	0.1116 mg/L	5.44 mg/L	3.44 mg/L
	Tube well water	0.104 mg/L	Not detected	Not detected
	Still water in fields	Not detected	5.64 mg/L	3.56 mg/L
	Drain water	Not detected	0.05 mg/L	0.033 mg/L

TABLE-4
COMPARISON OF RECORDED QUANTITIES OF PERSISTENT ORGANIC COMPOUNDS VEGETABLE AND FRUIT SAMPLES WITH STANDARDS

Sample	Sample name	Detected pesticides					
		Lahore		Rahim Yar Khan			
		Paraquat (mg/kg)	MRLs* (mg/Kg)	Difenoconazole (mg/kg)	MRLs* (mg/kg)	Bifenthrin (mg/kg)	MRLs* (mg/kg)
Fruit	Shakri Malta	9.265	0.02	209.31	0.80**	132.15	0.02
	Mango	5.0645	0.01	333.26	0.07	210	0.03
Vegetable	Pumpkin	40.395	0.07	515.1	0.2	325.29	0.40
	Lemon	12.07	0.02	433.16	0.80**	273.53	0.02

*Pesticides maximum residue limits Codex Standards; **WTO Canadian Standards.

high persistent organic compounds (pesticides) application frequency in the agricultural fields of both cities.

Use of persistent organic compounds in the form of pesticides has no doubt increased the agricultural yield but the presence of persistent residues of these compounds poses serious harmful environmental and health threats. Literature reveals that the presence of pesticides in fruits, vegetables, soil and water is alarming. The remains of persistent organic compounds in the form of pesticides have been accounted in different agricultural products all over the country [25-32]. The study highlights the tested fruits and vegetables from Rahim Yaar Khan are more contaminated than Lahore. In Pakistan, under Good Agricultural Practices no efforts have been made to determine the pesticide remains in agricultural supplies. Determination of residues of persistent pesticides should be legally recognized in developing countries like Pakistan to face the repercussion of WTO [33] and to manage the excessive application of the persistent pesticides in the country and to make certain the production of safe to use agricultural products by consumers.

Conclusion

The use of the unique combination of ethyl acetate and dichloromethane solvent system, sodium sulfate clean-up, C-18 Licospher column, Diode UV/visible detector set at 254 nm and mobile phase acetonitrile:water in the ratio of 40:60 with a flow rate of 1 mL/min of produces the complete separation of persistent organic compounds (pesticides) in samples generating a chromatogram free of co-eluting impurities. Additionally the assessment of the results with their relevant maximum residue limits unveil that all the tested vegetables and fruits have persistent organic compounds (pesticide) residual levels above maximum residue limits thus they are unfit for human consumption.

REFERENCES

1. S. Manahan, Environmental Chemistry, Lewis Publishers/CRC Press LLC, London, edn 7 (2000).
2. J. Buccini and H. Fiedler, The Development of a Global Treaty on Persistent Organic Pollutants (POPs), The Hand Book of Environmental Chemistry, Persistent Organic Pollutants, Springer-Verlag, Berlin/ Heidelberg, edn 3 (2003).
3. M.H. Wong, A.O.W. Leung, J.K.Y. Chan and M.P.K. Choi, *Chemosphere*, **60**, 740 (2005).
4. F. Mason and N. Barak, *Chemosphere*, **21**, 695 (1990).
5. G. Zhao, Y. Xu, G. Han and B. Ling, *Environ. Geochem. Health*, **28**, 341 (2006).
6. M.S. El-Shahawi, A. Hamza, A.S. Bashammakh and W.T. Al-Saggaf, *Talanta*, **80**, 1587 (2010).
7. Z. Parveen, M. Khuhro and N. Rafiq, *Bull. Environ. Contam. Toxicol.*, **74**, 170 (2005).
8. Z. Knezevic and M. Serdar, *Food Contr.*, **20**, 419 (2009).
9. F.A. Mansoor-ul-Hassan, M. Sagheer, M.F. Iqbal and M. Tariq, *Pak. Entomol.*, **27**, 53 (2005).
10. F.J. Arrebola, J.L. Martínez Vidal, M. Mateu-Sánchez and F.J. Álvarez-Castellón, *Anal. Chim. Acta*, **484**, 167 (2003).
11 M. Möder, P. Popp, R. Eisert and J. Pawliszyn, *Fresenius J. Anal. Chem.*, **363**, 680 (1999).
12. C. Ngan, U. Cheah, W.W. Abdullah, K. Lim and B. Ismail, *Water Air Soil Pollut. Focus*, **5**, 125 (2005).
13. T.K. Choudhury, K.O. Gerhardt and T.P. Mawhinney, *Environ. Sci. Technol.*, **30**, 3259 (1996).
14. K. Jinno, T. Muramatsu, Y. Saito, Y. Kiso, S. Magdic and J. Pawliszyn, *J. Chromatogr. A*, **754**, 137 (1996).
15. D. Clark, T. McElligott and E.W. Hurst, *Br. J. Ind. Med.*, **23**, 126 (1966).
16. A. Calderbank, Reviews of Environmental Contamination and Toxicology, Springer, pp. 71-103 (1989).
17. R. Frank, B.D. Ripley, H.E. Braun, B.S. Clegg, R. Johnston and T.J. O'Neill, *Arch. Environ. Contam. Toxicol.*, **16**, 1 (1987).
18. C. Wesseling, B.V.W. De Joode, C. Ruepert, C. León, P. Monge, H. Hermosillo and L.J. Partanen, *Int. J. Occup. Environ. Health*, **7**, 275 (2001).
19. S. Baskaran, R.S. Kookana and R. Naidu, *Pestic. Sci.*, **55**, 1222 (1999).
20. J.M. Hougard, S.P. Duchon, M. Zaim and P. Guillet, *J. Med. Entomol.*, **39**, 526 (2002).
21. I. Mukherjee, R. Singh and J. Govil, *Bull. Environ. Contam. Toxicol.*, **84**, 294 (2010).
22. S. Swart, M. Wingfield, W. Swart and G. Schutte, *Ann. Appl. Biol.*, **133**, 17 (1998).
23. X. Dong, L.Q. Ma, Y. Zhu, Y. Li and B. Gu, *Environ. Sci. Technol.*, **47**, 12156 (2013).
24. L. Samsøe-Petersen, E.H. Larsen, P.B. Larsen and P. Bruun, *Environ. Sci. Technol.*, **36**, 3057 (2002).
25. T. Anwar, I. Ahmad and S. Tahir, *J. Pak. Bot.*, **43**, 1133 (2011).
26. S.A. Baig, N.A. Akhtera, M. Ashfaq and M.R. Asi, *Am.-Eurasian J. Agric. Environ. Sci.*, **6**, 513 (2009).
27. S. Hussain, T. Masud and K. Ahad, *Pakistan J. Nutr.*, **1**, 41 (2002).
28. B.A. Khan, A. Farid, M.R. Asi, H. Shah and A.K. Badshah, *Food Chem.*, **114**, 286 (2009).
29. M.S. Khan, M.M. Shah, Q. Mahmood, A. Hassan and K. Akbar, *J. Chem. Soc. Pak.*, **33**, 816 (2011).
30. Y. Latif, S. Sherazi and M. Bhanger, *Ecotoxicol. Environ. Saf.*, **74**, 2299 (2011).
31. S.Z. Masud and N. Hasan, in ed.: M. Richardson, Pesticides Residues in Foodstuffs in Pakistan: Organochlorine, Organophosphorus and Pyrethroid Insecticides in Fruits and Vegetables, In: Environmental Toxicology Assessment, Taylor and Francis, London, pp. 269-279 (1995).
32. M.U. Tahir, S.I. Naik, S. Rehman and M. Shahzad, *Biomedica*, **25**, 171 (2009).
33. S. Tahir and T. Anwar, *Bull. Environ. Contam. Toxicol.*, **89**, 1138 (2012).