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Research Paper

Analysis of Chlorpyrifos Pesticide Residues in Surface Water, Ground Water and Vegetables through Gas Chromatography

Maryam Arain*, Khan Muhammad Brohi, Azizullah Channa, Rafi O Zaman Brohi, Sarmad Mushtaque, Kundan Kumar, Abdul Sameeu

Institute of Environmental Engineering & Management, MUET Jamshoro, PAKISTAN

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Abstract: This study was conducted in order to determine the residual concentration of Chlorpyrifos organophosphorus pesticide in surface water, ground water and vegetables (Okra, potato). From the eight union council (UCs) of tehsil Mehrabpur district Naushehro Feoz five samples of surface water, ground water and each vegetable were collected randomly. USEPA 3510C and USEPA 3620C the standard analytical methods were used for the extraction and clean-up of pesticide residues. In continuation to this the Gas Chromatography-Flame Ionization detector (GC-FID) was used to analyse the samples. Results revealed that in ground water samples the selected pesticide residual concentration was not more than maximum residual limit (MRL), the minimum value 6.6µg/L was found in UC2 while maximum was 11.2µg/L in UC5. Whereas, in vegetable samples the pesticide residual concentration in 20% of potato samples and 15% of okra samples were within MRL values and rest were found with higher concentrations. However, the higher residual concentration was observed in surface water samples having minimum and maximum values 43.46 µg/L and 79.7 µg/L, respectively. The presence of Chlorpyrifos pesticide residue a hazardous substance in surface water and vegetables samples pose harmful effect to the peoples who depend on these sources of water and food. Thus, it is desperately essential to conduct ongoing monitoring in recommended areas to regulate the use of such hazardous pesticide.

Keywords: *Gas chromatography, Chlorpyrifos, Vegetable, Surface Water, Ground Water,*

Introduction

The use of synthetic chemicals for pest control is the most reliable and fastest method of pest management. Worldwide to overcome the problems of lower yields as well as culturally and biologically occurring pests mostly the pesticides are applied in agricultural fields (Hester and Harrison, 2016). On the contrary the substantial adverse effects on ecology and environment the use of such hazardous and toxic pesticide is increasing serious public concern (Pujeri *et al*., 2015). Humans and other living organisms has a straight toxic effects due to the presence of these hazardous pesticides in drinking water and food (Eskenazi *et al*., 2008). Drinking water and the consumption of food such as cereals, vegetables and fruits the toxicity of these vulnerable chemical compounds to human and other animals can lead to many chronic and acute diseases including carcinogenic and mutagenic diseases (Damalas & Eleftherohorinos, 2011). During the last twenty years in Pakistan the use of pesticide 1, 18,579 metric tons in 2006 as opposed to 12,535 tons has seen an enormous surge. In Pakistan, major part of the pesticides used are insecticides, and its estimated that 20% of pesticide are sprayed on vegetables, fruits, paddy and sugarcane while the 80% of pesticides are used on cotton crops (Niaz *et al*., 2016). The predator pests' agro-ecological balance has disrupted due to the undifferentiated use of pesticide substances. In Pakistan since 1981 farmers becoming highly dependent on pesticides and due to entry of the private sector has also resulted a huge increase in pesticides applications (Masud & Hassan, 1995).

Pesticides are actually desirable but deteriorating the ecological balance. They pollute vegetation, turf, water, soil and kill living organisms. However, due to contamination there is a significant harm to human health as well. They have been known to damage environment in various ways (Ngowi *et al.*, 2001).

In both agricultural and non-agricultural fields, the organophosphorus insecticide chlorpyrifos is used widely. It is a neurotoxic substance which can kill various pests including worms and insects.

* *Corresponding: E-Mail: ms.maryam@faculty.muet.edu.pk; Tel: +923362202645*

Have high efficiency in insects killing, inexpensive and wide range of activities among other make them farmer's preferable choice (Rathod & Garg 2017). However, these toxic compounds have adverse effects on environment and human health. Due to acute effects on human health, according to world health organization (WHO) the chlorpyrifos pesticide has been classified as class II. The severe poisoning of chlorpyrifos leads to lung failure, unconsciousness, paralysis, suffocation and seizures. Intermediary exposure to chlorpyrifos leads to vomiting, diarrhea, weakness, impaired vision and neuromuscular symptoms like muscle spasms. Furthermore, increased saliva, sweating, eye watering and nausea are the result of short term exposure to chlorpyrifos. (Hussain and Siddique, 2010).

Therefore, the presence of chlorpyrifos in drinking water and vegetables creates serious threats to humans' health due to major environmental concerns in terms of surface water, ground water and soil contamination. Thus for providing better food chain there is a basic and prime need to determine the such hazardous compound in water and food sources (Beyer & Biziuk 2008). In this regards, the determination of residual concentration of chlorpyrifos has been carried out in surface water, ground water and vegetables (Okra and Potato) in the vicinity of tehsil Mehrabpur district Naushahro Feroze

Materials and Methods

Chemicals

In this study the chemicals used were chlorpyrifos ($C_9H_{11}Cl_3NO_3PS$), sodium sulphate (Na_2SO_4), florisil (MgO3Si) and n-hexane (99.9% pure HPLC grade). Chlorpyrifos purchased from Sun Crop Company Pakistan, n-hexane (99.9% pure HPLC grade) purchased from Daejung Chemicals & Metals Co Ltd, Seohaean-ro, Siheung-si, Gyeonggi-do, Korea, florisil purchased by Fluka AG, Buchs, Switzerland and sodium sulfate was purchased from Fisher Scientific UK limited, Bishop Meadow Road (Anila, 2013), Loughborough, UK

Sample collection and preservation

A total of 160 samples consists of 40 surface water, 40 ground water, 40 okras and 40 potatoes were collected and analysed in this study. The surface water samples, ground water samples and vegetables samples were collected from the agriculture fields of the 08 union councils of tehsil Mehrabpur district Naushahro Feroze. Five surface water samples from different regions of each union council were collected randomly after 24 hours of the spray of chlorpyrifos pesticide. After 15 days of spray the ground water samples were collected from tube wells and hand pumps near the crop fields of each union council. The clean polyethylene bottles were used for collecting water samples and clean polyethylene bags were used for the collection of vegetables at the time of harvesting. All the collected samples were properly labelled and were transported to the laboratory with the help of ice box and further preserved at 4°C in refrigerator before pesticides residue analysis.

Extraction and clean-up chlorpyrifos from water samples

The USEPA 3510C standard method was carried out for the extraction of chlorpyrifos from water samples. Methylene chloride or hexane was used as an extraction solvent and this is a liquid-liquid extraction method. 1 litter of sample was measured using a graduated cylinder and then 60 ml of hexane was added into separator funnel and shake for 1-2 min with periodic venting to release excess pressure. The upper organic layer was allowed to separate from sample for 10 min and collected in to Erlenmeyer flask. Using fresh portion of hexane solvent this process was repeated twice more. To remove water molecules from solvent it was passed from 2 grams of activated anhydrous sulphate which was activated by placing it for three hours at 400°C.

Similarly, for the clean-up of chlorpyrifos from ground water as well as surface water samples the 3620C USEPA florisil clean up method was used. Florisil was activated by heating overnight at 130°C by placing it in glass container with loosely covered aluminium foil. A 20mm inner diameter glass column filled with 2 grams of florisil was washed by using solvent and then hexane (extracted solvent) was passed through it. Then by rotary evaporator the solvent was pre-concentrated and finally 1-2 ml solvent was collected in vial and 1 µL was injected in to Gas Chromatograph (GC).

Extraction and clean-up of chlorpyrifos from vegetables samples

20 grams from each collected potato and okra sample was mixed with 20ml of distilled water. Then sample was crushed and grinded to homogenize with pastel and mortar. The Whatman filter

paper was used for filtering the sample. A 500 ml dilution of filtered sample was made and 30 ml of n-hexane was added in to separator funnel then shacked and allowed for ten minute for the separation of layers. For each sample the process was repeated twice. Furthermore, the water was then separated from organic layer and 2 grams of anhydrous sodium sulphate was added to it to dry organic layer. The USEPA 3620C florisil method was used for the cleaned up of organic layer.

Gas chromatograph analysis of Chlorpyrifos:

For the analysis of almost any organic compounds also for those that are non-volatile but can be turned into volatile derivatives most commonly the technique used is Gas chromatography (Hübschmann, 2015). The analysis of chlorpyrifos pesticide residue in all surface water, ground water and vegetables samples was carried out by first running standards of chlorpyrifos on GC-FID. The chlorpyrifos standard of 1000ppb was prepared and diluted with n-hexane up to different concentrations of 100, 80, 60, 40, and 20 ppb and calibration curve was drawn. The prepared sample after calibration were transferred into vials and by using micro syringe 1µL of each sample was injected into GC-FID. The concentration of chlorpyrifos pesticide in samples was then measured by linear equation(y= mx+C).

Gas chromatograph method:

In this study the Gas Chromatograph method proposed by Thermo Fischer Scientific Application Note 20705 was used. The equipment GC-2010 Plus, Shimadzu, Japan was used equipped with TRB-5 capillary column with 30 m length and 0.30 µm internal diameter, Flame ionized detector FID system with hydrogen generator and Nitrogen gas mobile were used during analysis of chlorpyrifos.

The FID set condition were as; hydrogen flow was 35 ml/min, air flow was 350 ml/min and nitrogen make up flow was 30 ml/min, respectively. For GC, initial oven temperature was 40°C hold for 1 min, the column flow of nitrogen mobile phase was 1.2 ml/min and the ramp temperature was 12°C/min raise up to final temperature 280°C and was hold for 10 min. Finally, using micro syringe (10 µL) the 1 µL of prepared sample volume was injected in to GC.

Results and Discussion

Analysis of standards of pesticide

Chlorpyrifos analysis was done with prepared standard solution of 1000ppb, diluted to the required concentration. Samples with varying concentration were inserted in the GC port by triplicate. Retention times gave the peaks of the standard solution of chlorpyrifos which were plotted with concentration to determine the calibration curve. The linear calibration curve can be seen in Figure 1

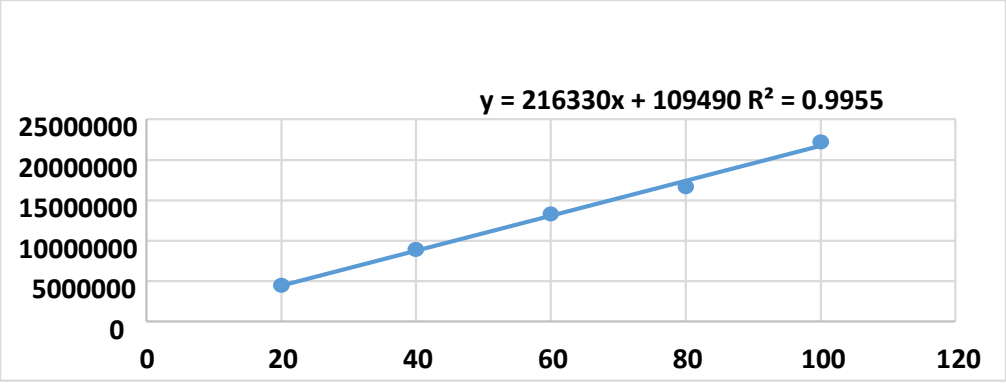

Figure 1. Calibration curve of chlorpyrifos pesticide

The equation of regression below (Eq.1) fits the data of the curve with the consideration of R^2 value as 0.984.

$$Y = 100.47\ X - 1058.8 \qquad (1)$$

The concentration of Chlorpyrifos pesticides was calculated by using above equation.

Analysis of vegetables samples

Okra samples

From the 8 union council of tehsil Mehrabpur district Naushahro Feroze the okra samples were collected and analysed, from 5 various regions of each union council 5 okra samples were analyzed by GC for chlorpyrifos pesticide. The chlorpyrifos pesticide residue concentration in 40 samples collected from 8 union councils are shown in Table 1. The maximum concentration of chlorpyrifos pesticide observed was 65.46 µg/kg in Union Council 5 (Kotri M. Kabir) and the minimum concentration observed in Union Council 2 (Mehrabpur 2) was 28.8 µg/kg.

Table 1. Shows the chlorpyrifos pesticide concentration in okra samples.

Union Council	Chlorpyrifos pesticides concentration (µg/kg)					Mean	STDEV
	1	2	3	4	5		
1. Mehrabpur 1	22.2	43.5	28.3	55	59	41.6	16.118
2. Mehrabpur 2	28	49.5	18	23	25.5	28.8	12.148
3. Halani	48.3	55.5	68.2	71.1	74	63.42	11.012
4. Behlani	42	33.3	38.4	52.8	61.7	45.64	11.479
5. Kotri.M.Kabir	55	63.6	73.5	58.7	76.5	65.46	9.2883
6. Lakha Road	33	36.4	52	42.2	36.7	40.06	7.4443
7. JaindoRajper	83.4	75.2	44.8	45.7	71.7	64.16	17.779
8. Saeed Pur	35	49.5	51.2	64.6	67	53.46	12.932

Potato Samples

The potato samples as like okra were also collected from the all union councils of tehsil Mehrabpur district Naushahro Feroze, from 5 different areas of each union council 5 potato samples were collected randomly and were analyzed by GC for chlorpyrifos pesticide. From 8 union councils the chlorpyrifos pesticide residual concentration in all samples are shown in Table 2. The chlorpyrifos pesticide maximum concentration was found as 66.32 µg/kg in Union Council 8 (Saeed pur) while the minimum concentration 28.68 µg/kg was observed in the sample from Union Council 1 (Mehrabpur 1).

Table 2 Shows the chlorpyrifos pesticide concentration in Potato.

Union Council	Chlorpyrifos pesticides concentration (µg/kg)					Mean	STDEV
	1	2	3	4	5		
1. Mehrabpur 1	29.4	34.6	25	36.2	18.2	28.68	7.339
2. Mehrabpur 2	31.2	41.1	33.7	53.5	56.2	43.14	11.332
3. Halani	33.6	44.2	28.5	21	23.9	30.24	9.148
4. Behlani	37.4	42.3	29.8	52.2	47.6	41.86	8.737
5. Kotri.M.Kabir	43.5	62.4	71.3	58.1	61.6	59.38	10.123
6. Lakha Road	45	38.6	41.5	33.7	29.2	37.6	6.259
7. JaindoRajper	52.6	48.9	59.3	45.7	46.2	50.54	5.610
8. Saeed Pur	87.2	78.5	65.2	48.3	52.4	66.32	16.613

Analysis of Water Samples

The water samples were collected from eight union councils of tehsil Mehrabpur, district Naushahro Feroze. The 5 surface water samples and 5 ground water samples randomly collected from the tube wells/hand pump near the crop fields, respectively.

Surface Water Samples

In surface water samples the chlorpyrifos pesticide was analyzed by GC. In all the 40 samples collected from 8 union councils the concentration of chlorpyrifos pesticide are shown in Table 3. In Union Council 5 (KotriM.Kabir) the maximum concentration 79.7 µg/L of chlorpyrifos pesticide was observed and the minimum concentration 43.46 µg/L was observed in the sample from Union Council 1 (Mehrabpur1).

Table 3. Shows the chlorpyrifos pesticide concentration in surface water samples

Union Council	Chlorpyrifos pesticides concentration (µg/L)					Mean	STDEV
	1	2	3	4	5		
1. Mehrabpur 1	36	45	63	32.5	40.8	43.46	11.906
2. Mehrabpur 2	70.5	55	63	67.5	48	60.8	9.2371
3. Halani	88	90	66.2	55	43.3	68.5	20.402
4. Behlani	53	72	81.5	66.2	47.8	64.1	13.770
5. Kotri.M.Kabir	83	91.2	68	76.8	79.5	79.7	8.492
6. Lakha Road	89.3	85	55	40.8	46.3	63.28	22.422
7. JaindoRajper	75.5	66	90.6	55.8	35.4	64.66	20.777
8. Saeed Pur	71	66.2	55.9	83.5	88.2	72.96	13.076

Ground Water Samples

From each union councils of tehsil Mehrabpur district Naushahro Feroze the ground water samples were collected and analyzed by GC. In all the samples collected from 8 union councils, the concentration of chlorpyrifos pesticide are shown in Table 4. In all ground water samples the values of pesticide residues were observed below the MRL guideline. The maximum concentration of chlorpyrifos pesticide 11.2 µg/L was observed in Union Council 5 (KotriM.Kabir) and minimum value 6.6 µg/L was found in the sample from Union Council 2 (Mehrabpur 2). The ground water samples were very less contaminated by chlorpyrifos because chlorpyrifos was absorbed by soil and does not readily leached from it, (WHO 2011).

Table 4. Shows the chlorpyrifos pesticide concentration in ground water samples

Union Council	Chlorpyrifos pesticides concentration (µg/L)					Mean	STDEV
	1	2	3	4	5		
1. Mehrabpur 1	9	15	13	7.5	5	9.9	4.068
2. Mehrabpur 2	7	10.5	4	8.5	3	6.6	3.110
3. Halani	5	12	9.5	6	11	8.7	3.074
4. Behlani	7	9	13.5	8	10	9.5	2.5
5. Kotri.M.Kabir	9.5	4.5	15	11	16	11.2	4.617
6. Lakha Road	13	7.5	9	5.5	12	9.4	3.110
7. JaindoRajper	8.5	5	12.5	15	5	9.2	4.480
8. Saeed Pur	16	5.5	6	10	12	9.9	4.364

Correlation study

The most familiar measure of dependence between two quantities is the Pearson product-moment correlation coefficient, or "Pearson's correlation coefficient", commonly called simply "The Correlation Coefficient". It is obtained by dividing the covariance of the two variables by the product of their standard deviations. Karl person developed the coefficient from a similar but slightly different idea by Galton.

Table 5 The Correlation chlorpyrifos pesticide in samples of Okra Vegetable

Union Councils	Mehrabpur1	Mehrabpur2	Halani	Behalani	Kotri. M.Kabir	Lakha Road	Jaindo Rainer	Saeed Pur
Mehrabpur 1	1							
Mehrabpur2	0.081	1						
Halani	0.707	529	1					
Behalani	-701	482	0.678	1				
Kotri.M.Kabir	-324	-0.253	0.675	0.309	1			
Lakha Road	122	554	0.530	-0.159	0.452	1		
Jaindo Rajper	-0.184	0.584	0.680	-0.095	256	892*	1	
Saeed Pur	0.918*	-0.242	0.928*	0.723	0.534	0.250	499	1

Correlation between the Chlorpyrifos residue concentration in Vegetable (Okra, Potato) in all 8 Union Councils of Taluka Mehrabpur

The results of correlation table 5 and Table 6 represented almost positive correlation between vegetable sample collected from 8 Union Councils but in okra and potato vegetable samples the -ve correlation was observed for the UC Mehrabpur 1 with UC Jaindo Rajper. While in potato samples only the correlation observed were union councils Behlani, Halani, Jaindo Rajper and Saeed pur.

Table 6 The Correlation chlorpyrifos pesticide in samples of Potato Vegetable

Union Councils	Mehrapur1	Mehrapur2	Halani	Behalani	Kotri. M.Kabir	Lakha Road	Jaindo Rajper	Saeed Pur
Mehrabpur 1	1							
Mehrabpur2	-0.135	1						
Halani	0.329	-556	1					
Behalani	0.239	0.879*	-375	1				
Kotri.M.Kabir	-0.237	0.189	-0.029	-0.204	1			
Lakha Road	0.303	0.977**	0.530	0.769	-0.322	1		
Jaindo Rajper	-0.220	0.810	0.137	0.960**	0.263	0.717	1	
Saeed Pur	0.208	0.839	0.817	-0.586	-0.470	0.840	0.396	1

Correlation between the Chlorpyrifos residue concentration in Water Sample (Surface, Ground) in all 8 Union Councils of Taluka Mehrabpur

The correlation tables 7 and Table 8 represent that in surface and ground water samples the negative correlation for chlorpyrifos concentration observed in union councils Mehrabpur 1, Behlani, Lakha road, Jaindo Rajper and Saeed Pur.

Table 7 The Correlation chlorpyrifos pesticide in samples of Surface Water

Union Councils	Mehrapur1	Mehrapur2	Halani	Behalani	Kotri. M.Kabir	Lakha Road	Jaindo Rajper	Saeed Pur
Mehrabpur 1	1							
Mehrabpur2	-0.314	1						
Halani	0.067	0.182	1					
Behalani	0.701	0.200	0.263	1				
Kotri.M.Kabir	-0.502	-0.265	0.552	-0.325	1			
Lakha Road	-0.024	-0.027	0.943*	-0.026	0.658	1		
Jaindo Rajper	0.617	0.409	0.612	0.699	-0.313	0.425	1	
Saeed Pur	-0.785	-0.078	-0.628	-0.770	0.184	-0.471	-931	1

Table 8/ The Correlation chlorpyrifos pesticide in samples of Ground Water

Union Councils	Mehrapur 1	Mehrapur 2	Halani	Behalani	Kotri. M.Kabir	Lakha Road	Jaindo Rajper	Saeed Pur
Mehrabpur 1	1							
Mehrabpur2	0.485	1						
Halani	0.357	-0.107	1					
Behalani	0.326	-0.579	0.520	1				
Kotri.M.Kabir	0.584	0.946*	-0.021	0.522	1			
Lakha Road	-0.347	0.561	-0.089	-0.080	0.319	1		
Jaindo Rajper	-0.053	.016	-0.616	0.139	0.248	0.527	1	
Saeed Pur	-0.712	-0.220	0.664	0.636	0.178	0.680	-0.088	1

Conclusions

This study concluded that the residue of chlorpyrifos pesticide in majority of vegetables (okra and potato) samples collected from all UCs of tehsil Mehrabpur district Naushehro feroz crossed the MRL value 30 µg/L. The okra and potato samples were highly contaminated with chlorpyrifos, the maximum concentration 65.46 µg/L was observed in the okra sample of union council 5 Kotri. M. Kabir and 66.32µg/L in potato sample of union Council 8 Saeed pur. Results indicates that all the vegetables were found with higher concentration of Chlopyrifos and only 20 % of potato samples and 15% of okra samples contained chlorpyrifos residual concentrations within MRL values. In all samples of surface water, the residual concentration of chorpyrifos is above the MRLs values because of direct contact of pesticide with surface water. The maximum value of 79.7 µg/L was observed in union council 5 Kotri M.Kabir. Whereas, for the ground water samples the maximum concentration of chlorpyrifos pesticide observed was 11.2 µg/L in union council 5 Kotri. M. Kabir and rest were also below the MRL s guideline value

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