Pakistan J. Zool., vol. 46(1), pp. 231-236, 2014.

Gas Chromatographic Analysis of Pesticide Residues in Soil of Bahawalpur District, Punjab, Pakistan*

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Abstract.- Residues of organophosphate (OP) and organochlorine (OC) pesticides were monitored in soil samples collected from cotton growing areas in Bahawalpur district, Punjab, Pakistan. All the 12 soil samples were found contaminated with dichlorvos, dimethoate, methyl parathion, fenitrothion, endosulfan, mevinphos, chlorpyriphos and profenofos. The most widely detected pesticide was mevinphos and was found in eleven samples with mean concentration of 0.199 mg Kg^{-1}. Endosulfan was the second most often detected pesticide investigated in ten samples with the mean concentration of 0.262 mg Kg^{-1}. fenitrothion was the third most detected pesticide in seven samples with mean concentration of 0.3 mg Kg^{-1}. Profenofos was not detected in any soil sample. The mean value of ∑pesticides was found to be 1.175 ± 0.014 mg Kg^{-1} at 95% confidence level in soil samples collected with highest value of 3.161 mg Kg^{-1} in sample MS-3 followed by 2.527 mg Kg^{-1} in MS-2, while, other samples (83%) were found contaminated usually at concentration less than 2 mg Kg^{-1}

Key words: Pesticides residues, organophosphate, organochlorine, endosulfan, profenofos

INTRODUCTION

In Pakistan, although efforts have been made to solve the challenges of dry lands (Rashid *et al*., 2004), to study the impact of soil on vegetation (Awan *et al.*, 1992), reclamation and management of alkaline soil (Gupta and Zia, 2002) and moisture conservation in soil (Salim *et al*., 2000) during the past decade but little has been done on the fate of pesticides in soil. Baig (1985) and Hussain *et al*. (1988) have reported DDT residues in agricultural soil of Punjab, Khyber Pukhtunkhwa and Baluchistan. This contamination was present on top 5 cm layer of sandy loam soils. Jabbar *et al*. (1993) reported monocrotophos, cyhalothrin, dimethoate, fenvalerate, cypermethrin and profenofos in the top layer soil of Samundari, a cotton growing area. Tahir *et al*. (1999) analyzed the fortified soil samples and reported 100%, 67% and 94% recoveries of methyl parathion at spiking level of 0.17, 1.7 and 8.7 μgL^{-1}, respectively. The data showed the evidence that capillary column GC-ECD could be used reliably and advantageously for pesticide analysis. Recently, Anwar *et al*. (2012) reported the chlorpyriphos as the most widely detected pesticide with mean concentration of 0.486 mg Kg^{-1} in soil samples collected from the agricultural areas of Nawabshah District, Sindh. Shegunova *et al*. (2007) screened the organochlorine (OC) in soil and biotic samples from the Czech Republic although these pesticides had never been used in large quantities in this region. Their residues were found to be persisting in the top layer soil. Concentration of pesticides was found to be higher in the mountains regions than those in agricultural areas and detection of traces of pesticides in the region supported on the occurrence on soil due to the atmospheric redistribution rather than as a result of direct application. Wang and Zhang (2006) extracted 13 OC insecticides in soil with an ordinary pressure Microwave with Assisted Extraction (MAE) system and determined by GC. Detection limits for different substances were 0.033-0.853 ng g^{-1}. The pesticides accumulated in the soil for relatively longer period of time and then passed into various parts of the plant grown on the contaminated soil (Malhat *et al*., 2012). Tariq *et al*. (2010) studied the fate of pesticides in sandy loam soil of Pakistan at different water table depths. Ahad *et al*. (2010) reported the OC pesticide in the soil

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0030-9923/2014/0001-0231 $ 8.00/0

samples collected from the obsolete pesticide stores from three provinces (Punjab, Sindh and KPK) of Pakistan. A review on occurrence and levels of OC contaminants in the different environmental compartment of Pakistan has been recently carried out by Ali *et al*. (2012). The present investigation was carried out to monitor the residues in agricultural soil where heavy pesticides are being used to develop the strategy to prevent adverse effects on public health (Ahmad, 1998) and farmers' community (Khan *et al*., 2010).

MATERIALS AND METHODS

Sampling

All the samples were collected randomly from the selected cotton growing areas of Bahawalpur District, Punjab, Pakistan. The field study was limited to manageable geographical areas where farmers and female cotton pickers were living and have a great potential to be exposed to pesticides. Twelve soil samples were collected from different agricultural fields of Moza Salah (MS), Dera Bhaka (DB) and Abbas Nagar (AN) and analyzed for the residues of dichlorvos, mevinphos, dimethoate, methyl parathion, fenitrothion, chlorpyriphos, endosulfan and profenofos. The soil samples were collected in plastic bags and transported to laboratory for analysis. The method reported by Tahir *et al*. (1999) was followed for the extraction of pesticide residues in soil. Fifty grams of soil sample were taken in a conical flask and then 150 ml of a mixture of acetone: hexane (1:1) was added. This was shaken for 1 hour with the help of mechanical shaker at a rate of about 300 Osc/minute. The mixture was filtered through a glass wool plug with Whattman filter paper No. 542 into a separating funnel. The extract was washed with distilled water (2×100 ml). The lower aqueous layer was discarded and a few grams of anhydrous sodium sulphate were added. Twenty ml of the aliquot was transferred to round bottom flask and evaporated to dryness at 40°C in a rotary evaporator. The contents of the flask were reconstituted in 6 ml ethylacetate and cyclohexane (1:1) mixture and then passed through high flow super cells. Two ml of this sample was applied on Gel Permeation Chromatograph (GPC) for further cleanup. After passing through GPC column, the samples were again dried under vacuum and reconstituted in 1ml ethylacetate for analysis on GC.

Experimental

Apparatus

Gas Chromatograph, Perkin-Elmer, Autosystem, Microprocessor fitted with Electron Capture Detector (ECD-Ni^{63}) and Nitrogen Phosphorous Detector (NPD). Nitrogen and Air Generator Peak Scientific. Hydrogen Generator, Peak Scientific, Gel Permeation Chromatograph (GPC), Mikrolab Arhus A/S, USA. Rotary Evaporator, made Buchi R-114/A, Switzerland. Food Blender, Germany. FlASK Shaker SF1, Sartatorius single pan analytical balance and Refrigerator/Freezer.

Reagents

The analytical grade standards of insecticides (dichlorvos, dimethoate, methyl parathion, fenitrothion, endosulfan, mevinphos, chlorpyriphos and profenofos were purchased form Riedel-d Haen AG Seelze, Germany or obtained from other institutes of Pakistan. Stock solutions and required working dilutions were prepared in ethlylacetate . All other solvents and reagents were of extra pure GC/HPLC grade. Acetone, Ethylacetate, anhydrous sodium sulphate, sodium hydroxide, potassium dichromate, sodium chloride, (Merk Company), cyclohexane and n-Hexane, high flow super cells, (British Drug House Company). dichloromethane (Lock-light Ltd.), propane 1, 2-diol (Pharmacos Ltd., UK), bio-beads, SX3 200-400 (Reidel-de Haën) and Millipore distilled water.

Instrumentations

Multi residue method for analysis of soil was developed by using Gas Chromatograph (GC) equipped with Electron Capture Detector (ECD). All the pesticides could be determined simultaneously under the conditions. Retention time and calibration curve of each standard insecticide was prepared as mentioned by Anwar *et al*. (2012) .

Quality control and safety

All general laboratory safety rules for sample preparation and analysis were followed as reported by Anwar *et al*. (2012).

RESULTS AND DISCUSSION

The quantity of pesticide residues detected in the samples collected from different locations of Nawabshah is reported in Table I. A total of 12 samples were collected from different location of Moza Salah (MS), Dera Bhaka (DB) and Abbas Nagar (AN) and analyzed for the residues of dichlorvos, mevinphos, dimethoate, methyl parathion, fenitrothion, chlorpyriphos, endosulfan and profenofos.

The average values (in $mg.kg^{-1}$) of pesticide residues in soil sample MS-1 were 0.318 dichlorvos, 0.002 mevinphos, 0.038 dimethoate, 0.001 methyl parathion, 0.915 endosulfan and 0.495 profenofos. However, residues quantified in sample MS-2 were 0.062 dichlorvos, 0.0450 dimethoate, 0.045 methyl parathion, 0.985 fenitrothion and 0.985 mg kg^{-1} chlorpyriphos. In soil sample MS-3 the residues of dichlorvos was 0.653, mevinphos 0.750, methyl parathion 0.007, fenitrothion 0.635, endosulfan 0.766 mg and profenofos 0.350 mg kg^{-1}. In soil sample MS-4 the residues of mevinphos, dimethoate, fenitrothion and endosulfan were found in quantity of 0.150, 0.740, 0.005 and 0.930 $mgkg^{-1}$, respectively.

The average values (mg kg^{-1}) of pesticide residues determined in soil sample DB-1 were 0.066 dichlorvos, 0.080 mevinphos and 0.049 endosulfan. However, residues quantified in sample DB-2 was mevinphos 0.120, methyl parathion 0.007, fenitrothion 0.475 and endosufan 0.052 mg kg^{-1}. In soil sample DB-3 the residues of mevinphos 0.150, dimethoate 0.001, fenitrothion 0.045 and endosulfan 0.045 mg kg^{-1}. In soil sample DB-4 the residues of dichlorvos, mevinphos and endosulfan were found in quantity of 0.480, 0.630, 0.345 mg kg^{-1}, respectively.

The average values of pesticide residues determined in soil sample AN-1 were 0.0076 mevinphos and 0.015 mg kg^{-1} endosulfan. However, residues quantified in sample AN-2 as mevinphos (0.355) and fenitrothion (0.985 mg kg^{-1}). In soil sample AN-3 the residues of dichlorvos (0.130), mevinphos 00.025, dimethoate 0.020, fenitrothion 0.470 and endosulfan 0.001 mg kg^{-1}. In soil sample AN-4 the residues of mevinphos, dimethoate, methyl parathion and endosulfan were found in the quantity of 0.051 $mgkg^{-1}$, 0.075 $mgkg^{-1}$, 0.045 mg kg^{-1} and 0.024 mg kg^{-1}, respectively.

Most of the soil samples collected from Bahawalpur district was found contaminated with pesticide residues at different levels. The most widely detected pesticide was mevinphos and was found in eleven samples with mean concentration of 0.199 mg Kg^{-1}. Endosulfan was the second most often detected pesticide investigated in ten samples with the mean concentration of 0.262 mg Kg^{-1}. Fenitrothion was the third most detected pesticide in seven samples with mean concentration of 0.3 mg Kg^{-1}. Profenofos was not detected in any soil sample. The mean values of ∑pesticides was found to be 1.175 ± 0.014 mg Kg^{-1} at 95% confidence level in soil samples collected with highest value of 3.161 mg Kg^{-1} in sample MS-3 followed by 2.527 mg Kg^{-1} in MS-2, while, other samples (83%) were found contaminated usually at concentration less than 2 mg Kg^{-1}.

Presently the pesticides used are mostly synthetic organic compounds. The sediments might act as an important sink for persistent organic pollutants including many pesticides used presently or in the past. They are relatively insoluble in water and are retained strongly by the soil. Soil acts as filter buffer and degradation of pollutants with respect to storage of pollutants with the help of soil organic carbon (Burauel and Bassmann, 2005). Soil acts as a pathway of pesticide transport to contaminate water, plants, food and effect on human via runoff, leaching, transfer of mineral nutrients and pesticides from soil into the plants and animals that constitute human food chain (Abraham, 2002). Persistent pesticides slowly break down into the soil and lead to contamination which is closely correlated to human activities like industrial discharge, agricultural applications and deforestation which leads to soil erosion (Bhattacharya *et al*., 2003).

In the present study the soil samples analyzed were collected from the major cotton growing areas of Bahawalpur district Punjab. Most of the soil samples collected from these areas was contaminated with pesticides. The most widely detected pesticides which are currently or have been used heavily in the past in Bahawalpur included mevinphos, endosulfan and fenitrothion in 92%,

Table I.- Pesticide residues (mg kg^{-1}) in soil samples collected from Bahawalpur, Punjab, Pakistan.

Locations*	Dichlorvos	Mevinphos	Dimethoate	Methyl Parathion	Fenitrothion	Chlorpyrifos	Endosulfan	Profenofos	Total
MS-1	0.318	0.002	0.038	0.001	0	0	0.915	0.495	1.769
MS-2	0.062	0	0.45	0.045	0.985	0.985	0	0	2.527
MS-3	0.653	0.75	0	0.007	0.635	0	0.766	0.35	3.161
MS-4	0	0.15	0.74	0	0.005	0	0.93	0	1.825
DB-1	0.066	0.08	0	0	0	0	0.049	0	0.195
DB-2	0	0.12	0	0.007	0.475	0	0.052	0	0.654
DB-3	0	0.15	0.001	0	0.045	0	0.045	0	0.241
DB-4	0.48	0.63	0	0	0	0	0.345	0	1.455
AN-1	0	0.076	0	0	0	0	0.015	0	0.091
AN-2	0	0.355	0	0	0.985	0	0	0	1.34
AN-3	0.13	0.025	0.02	0	0.47	0	0.001	0	0.646
AN-4	0	0.051	0.075	0.045	0	0	0.024	0	0.195
Mean	0.142	0.199	0.110	0.009	0.300	0.082	0.262	0.070	1.175
STD	0.221	0.249	0.236	0.017	0.395	0.284	0.381	0.167	1.008
95% confide.	0.004	0.005	0.004	0.000	0.007	0.005	0.007	0.003	0.014

Note: Values are the mean of duplicate samples. Whereas, 0 stands for "pesticide not detected" and it is included in calculating the mean.
*MS, Moza Salah; DB, Dera Bhaka; AN, Abbas Nagar.

83% and 58% soil samples respectively, followed by 50% soil samples contaminated with dimethoate and dichlorovs. On the other hand, Anwar (2009) reported mevinphos in seven soil samples and endosulfan and fenitrothion in all soil samples of Lodhran, Punjab. These results from cotton growing areas were in good agreement with data from the previous investigations. Endosulfan an OC pesticide was reported as pesticide of the particular concern in sediments from Queensland irrigation areas above the environmental guidelines (0.01 μgL^{-1}) (Simpson, 1998). The sediments collected from the eleven areas in Queensland regions dominated by cotton cultivation were found contaminated with pesticides used presently and in the past. The most often detected pesticide was endosulfan which was detected in 78 samples out of 103 (Muller *et al*., 2000). Endosulfan due to its persistency and commonly used feature on crop was frequently found when applied in the past in the sediments. Pesticides such as endosulfan appeared widely distributed in soil from three locations of cotton growing areas of Pakistan. The pesticides retained for a longer period of time in the soil, pass into various parts of plants grown on the contaminated soil (Anwar, 2009, 2011).

Several studies (Singh *et al*., 2005; Gao *et al*., 2005) have reported the detection of pesticides in soil and the most frequent pesticides detected were of OC group which is more persistent, stay in the sediment, decompose very slowly and persist for several years as they are insoluble in water and are retained by the soils. DDT was detected in most of the soil samples. Baig (1985) reported DDT in organic soil of Punjab and NWFP, while the most applied DDT was retained on top 5 cm layer in sandy loam soil (Hussain *et al*., 1988; Jabbar *et al*., 1993).

In Pakistan the presence of pesticide residues was reported by several researchers (Baig, 1985; Hussain *et al*., 1988; Bano and Siddiqui, 1991; Jabbar *et al*., 1993; FAO, 2001; Sanpera *et al*., 2002; Tariq *et al*., 2006; Anwar, 2009; Anwar *et al*., 2011, 2012). The detection of pesticides in soil and their findings are in full agreement with the present results where the mevinphos, endosulfan and fenitrothion, were detected in most of the samples. Mevinphos was found in 11 samples out of 12 from Bahawalpur (present studies). Jabbar *et al*. (1993) analyzed the soil of Samundari area of Faisalabad district in Punjab, Pakistan for pesticide residues. All the studied soil samples were found

contaminated with varying amount of different pesticide residues, while the monochrotophos, dimethoate and profenofos were found at one foot depth and DDT and its metabolites were detected at three feet depth in traces. These results are not in complete agreement with the present results where the soil collected from different locations of cotton growing areas of Bahawalpur were found contaminated with mevinphos, endosulfan, fenitrotion and dimethoate in most of the collected samples but only a few samples were found contaminated with profenofos. In the present study monochrotphos was not monitored because it was not reported to be used in the Bahawalpur district. Tariq *et al*. (2004, 2006) studied the hydrophobicity and persistence of pesticides that controlled the accumulation in different soil series of Pakistan. It was observed that less water soluble (<1 mg L^{-1}) pesticides have the potential to accumulate in sediment and aquatic biota, which is in full agreement with present study that most pesticides used on cotton are insoluble in water so that they persist in the soil without any influence of temperature, humidity and microbial activity on degradation and persistence of commonly used cotton pesticides in sandy loam soils of the cotton growing areas of Pakistan, specially Punjab (Tariq *et al*., 2006). Pesticides that breakdown rapidly are not likely to be detected in groundwater. Some pesticides like OC decompose very slowly and may persist for years and are retained by soil due to their insolubility in water. In the present study the detection of pesticides in soil from different locations demonstrated the difference in pesticide residues that could be related to the cultivation of crop with different time intervals and pesticide usage like chlorpyrifos was detected only in one sample of Bahawalpur as compared to 16 samples out 19 from Nawabshah district (Anwar *et al*., 2012) and in eight samples collected from Lodhran out of twelve soil samples (Anwar, 2009). It is concluded that in order to prevent adverse effects on public health the dissipation studies on pesticides must be carried out in different climatic zone of agriculture in Pakistan to calculate the half life and pre-harvest interval especially in vegetables growing on pesticide contaminated soil.

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(Received 18 April 2012, revised 4 November 2013)