

Determination of Pesticide Residues in Soil of Nawabshah District, Sindh, Pakistan*

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Abstract.- Pesticide residues of organophosphate (OP) and organochlorine (OC) of most commonly used classes were monitored in soil samples collected from cotton growing areas in Nawabshah district, Sindh. All the 19 soil samples presently analyzed were found contaminated with used pesticides (*i.e.* dichlorvos, dimethoate, methyl parathion, fenitrothion, endosulfan, mevinphos, chlorpyrifos and profenofos) and the varying degree of concentration and frequency were found in the top soil. The most widely detected pesticide was chlorpyrifos found in 16 samples with mean concentration of 0.486 mg kg⁻¹. Endosulfan was the second most often detected pesticide investigated in 15 samples containing the mean concentrations of 0.426 mg kg⁻¹. Dimethoate was the third most detected pesticide in 14 samples with mean concentration of 0.555 mg kg⁻¹.

Keywords Pesticide residues in soil, organophosphate, pyrethroid, organochlorine.

INTRODUCTION

According to several researchers a range of pesticides residues have been found in the soil and DDT residues have been reported in agricultural soil environment of Punjab and NWFP and contamination of paddy ecosystem with DDT (Baig, 1985). DDT sprayed in Baluchistan (Hussain *et al.*, 1988) showed DDT on top 5 cm layer in sandy loam soil, Jabbar *et al.* (1993) reported the monocrotophos, cyhalothrin, dimethoate, fenvalerate, cypermethrin and profenofos in the top 30 cm soil of Samudari, a cotton growing area. Tahir *et al.* (1999) analyzed the fortified soil samples recoveries on GC-ECD.

It was found that % recoveries of methyl parathion were 100%, 67% and 94% at spiking level of 0.17, 1.7 and 8.7 µg L⁻¹. The data showed the evidence that capillary column GC-ECD could be used reliably and advantageously for pesticide analysis. Shegunova *et al.* (2007) screened the OC in soil and biotic samples from the Czech, Republic although these pesticides had never been used in large quantities in this region. Results indicated that their residues were found to be persisting in the top

layer soil concentration in the mountains generally higher 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. Tor *et al.* (2006) demonstrated that ultrasonic solvent extraction of organochlorine pesticides from soil including α, β, γ and Δ-HCH, heptachlor, aldrin, o, p-DDE and p-DDT, methoxychlor, mixer from soil was optimized and detection was efficiently carried out by GC equipped with ECD. Twice ultrasonic extraction using 25 ml of a mixture of petroleum ether and acetone (1:1) for 20 minutes of sonication showed satisfactory extraction efficiency. Recoveries of pesticides from fortified soil samples were over 80% for 3 different fortification levels between 15-200 µg Kg⁻¹ and relative standard deviations of the recoveries were generally below 6%. Ultrasonic solvent extraction made the procedure most rapid between the use of time in ultrasonic extraction and considerably reduced compound when flask was not shaken during soxhlet extraction. Wang and Zhang (2006) extracted 13°C in soil with an ordinary pressure microwave with assisted extraction (MAE) system and determined by GC detection limits for different substances which were 0.033-0.853 ng g⁻¹. The pesticides accumulated in the soil for relatively longer period of time and then

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passed into various parts of the plant grown on the contaminated soil. It is concluded that to prevent adverse effects on public health it is a must to carryout regular monitoring system of these kinds for the establishment of safety measures (Ahmad, 1998)

MATERIALS AND METHODS

Sampling

All the samples were collected randomly from the selected cotton growing areas in districts of Nawabshah from upper Sindh. 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. Nineteen (19) soil samples were collected from different agricultural fields of Sakrand, Nawabshah and Daulatpur of Nawabshah district, Sindh. 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 Whatman 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-Ni⁶³) 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, Sartorius single pan analytical balance and Refrigerator/Freezer.

Reagents

The analytical grade standards of insecticides (dichlorvos, dimethoate, methyl parathion, fenitrothion, endosulfan, mevinphos, chlorpyrifos and profenofos) were purchased from Riedel-de Haen AG Seelze, Germany or obtained from other institutes of Pakistan. Stock solutions and required working dilutions were prepared in ethylacetate. All other solvents and reagents were of extra pure GC/HPLC grade. Acetone (Merk) Ethylacetate (Merk), cyclohexane (BDH) and n-Hexane (BDH). anhydrous sodium sulphate (Merk), dichloromethane (Lock-light Ltd.), propane 1, 2-diol (Pharmacos Ltd., UK), high flow super cells (BDH) bio-beads, SX3 200-400 (Reidel-de Haën), sodium hydroxide (Merck), potassium dichromate (Merck), sodium chloride (Merck) 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 for each standard insecticide was determined. Then calibration curve of each standard insecticide was prepared by using computer software Turbocrome[®] made by PE Nelson company, USA and the limit of detection was calculated by DOS based computer software Super Cal-5 (Unpublished).

Quality control and safety

All general laboratory safety rules for sample preparation and analysis were followed. The calibration standards were added to matrix blank extract to correct for matrix background interference. All standards were kept in refrigerator. The expiration date of each mixed working standard was six months from the preparation date. The recoveries of the matrix spike were within the

control limits. The retention time was within $\pm 2\%$ of that of the standard. The method validation consisted of three sample sets. Each set included three levels of fortification (0.01, 0.05 and 1.0 ppb) and a method blank. All spikes and method blank samples were processed through the entire analytical method. Quantification was based on external standard calculation using the peak area. The software used a linear curve fit at all levels weighed equally. For responses within calibration range, comparison of the retention time of the peaks with retention time of standards was made. When the sensitivity of detector decreased due to late eluting compounds it was managed with changing the injector liner and trimming the column. The limits of detection (LOD) of pesticides were calculated from 0.01-0.08 $\mu\text{g ml}^{-1}$.

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 19 samples were collected from different location of Sakrand (SK), Daulatpur (DP) and Nawabshah (NS) and analyzed for the residues of dichlorvos, mevinphos, dimethoate, methyl parathion, fenitrothion, chlorpyrifos, endosulfan and profenofos.

In soil sample SK-1 the residues of dichlorvos, mevinphos, dimethoate, fenitrothion, chlorpyrifos and endosulfan were found in the quantity of 0.024 mg kg^{-1} , 0.55 mg kg^{-1} , 1.35 mg kg^{-1} , 0.049 mg kg^{-1} , 1.22 mg kg^{-1} and 1.11 mg kg^{-1} respectively. In soil sample SK-2 the residues of dichlorvos, mevinphos, dimethoate, fenitrothion, chlorpyrifos and endosulfan were found in the quantity of 0.01 mg kg^{-1} , 0.825 mg kg^{-1} , 1.58 mg kg^{-1} , 0.2 mg kg^{-1} , 1.42 mg kg^{-1} and 0.354 mg kg^{-1} respectively. In soil sample SK-3 the residues of dichlorvos, mevinphos, dimethoate, fenitrothion, chlorpyrifos and endosulfan were found in the quantity of 0.014 mg kg^{-1} , 0.65 mg kg^{-1} , 0.64 mg kg^{-1} , 0.004 mg kg^{-1} , 0.575 mg kg^{-1} and 0.628 mg kg^{-1} respectively. In soil sample SK-4 the residues of dichlorvos, mevinphos, dimethoate, fenitrothion, chlorpyrifos and endosulfan were found in the quantity of 0.016 mg kg^{-1} , 0.74 mg kg^{-1} , 0.032 mg

kg^{-1} , 0.42 mg kg^{-1} , 1.35 mg kg^{-1} and 0.331 mg kg^{-1} respectively. In soil sample SK-5 the residues of dichlorvos, mevinphos, dimethoate, fenitrothion, chlorpyrifos and endosulfan were found in the quantity of 1.335 mg kg^{-1} , 0.735 mg kg^{-1} , 0.001 mg kg^{-1} , 0.415 mg kg^{-1} and 0.626 mg kg^{-1} respectively.

In soil sample DP-1 the residues of mevinphos, dimethoate, methyl parathion, fenitrothion, chlorpyrifos, endosulfan and profenofos were found in the quantity of 0.001 mg kg^{-1} , 0.041 mg kg^{-1} , 0.001 mg kg^{-1} , 0.591 mg kg^{-1} , 0.001 mg kg^{-1} , 0.746 mg kg^{-1} and 0.002 mg kg^{-1} respectively. In soil sample DP-2 the residues of dichlorvos, mevinphos, dimethoate, methyl parathion, fenitrothion, chlorpyrifos, endosulfan and profenofos were found in the quantity of 0.06 mg kg^{-1} , 0.315 mg kg^{-1} , 0.62 mg kg^{-1} , 0.003 mg kg^{-1} , 0.002 mg kg^{-1} , 0.363 mg kg^{-1} , 0.85 mg kg^{-1} and 0.001 mg kg^{-1} , respectively. In soil sample DP-3 the residues of dichlorvos, mevinphos, dimethoate, methyl parathion, fenitrothion, chlorpyrifos, endosulfan and profenofos were found in the quantity of 0.071 mg kg^{-1} , 0.012 mg kg^{-1} , 0.515 mg kg^{-1} , 0.011 mg kg^{-1} , 0.230 mg kg^{-1} , 0.021 mg kg^{-1} , 0.624 mg kg^{-1} and 0.014 respectively. In soil sample DP-4 the residues of dichlorvos, mevinphos, dimethoate, methyl parathion, fenitrothion, chlorpyrifos, endosulfan and profenofos were found in the quantity of 0.065 mg kg^{-1} , 0.516 mg kg^{-1} , 0.843 mg kg^{-1} , 0.061 mg kg^{-1} , 0.001 mg kg^{-1} , 0.014 mg kg^{-1} , 0.948 mg kg^{-1} and 0.016 respectively. In soil sample DP-5 the residues of dichlorvos, mevinphos, dimethoate, methyl parathion, fenitrothion, chlorpyrifos, endosulfan and profenofos were found in the quantity of 0.001 mg kg^{-1} , 0.620 mg kg^{-1} , 0.176 mg kg^{-1} , 0.395 mg kg^{-1} , 0.0323 mg kg^{-1} , 0.65 mg kg^{-1} , 0.073 mg kg^{-1} , 0.046 mg kg^{-1} , respectively. In soil sample DP-6 the residues of dichlorvos, mevinphos, dimethoate, fenitrothion, chlorpyrifos, endosulfan and profenofos were found in the quantity of 0.001 mg kg^{-1} , 0.27 mg kg^{-1} , 0.255 mg kg^{-1} , 0.65 mg kg^{-1} , 0.002 mg kg^{-1} , 0.98 mg kg^{-1} and 0.03 mg kg^{-1} respectively. In soil sample DP-7 the residues of dichlorvos, mevinphos, dimethoate, methyl parathion, fenitrothion, chlorpyrifos, endosulfan and profenofos were found in the quantity of 0.001 mg kg^{-1} , 0.43 mg kg^{-1} , 0.915 mg kg^{-1} , 0.022 mg kg^{-1} ,

Table I.- Pesticide residues (mg kg⁻¹) in soil samples collected from Nawabshah, Sindh, Pakistan.

S. No.	Sample codes	Dichlorvos	Mevinphos	Dimethoate	Methyl parathion	Fenitrothion	Chlorpyrifos	Endosulfan	profenofos	Total
1	SK-1	0.024	0.55	1.35	0	0	1.22	1.11	0	4.254
2	SK-2	0.01	0	1.58	0	0.2	1.42	0.35	0	3.56
3	SK-3	0.014	0.65	0	0	0	0.575	0.62	0	1.859
4	Sk-4	0	0.75	0.032	0	0.42	1.35	0.33	0	2.882
5	SK-5	0	0.735	0	0	0.415	0	0.62	0	1.77
6	DP-1	0	0	0.041	0.001	0	0.001	0.746	0.002	0.791
7	DP-2	0.062	0	0.62	0.003	0	0.363	0.855	0	1.903
8	DP-3	0	0.012	0.515	0	0.23	0.021	0	0.014	0.792
9	DP-4	0.065	0.516	0.843	0.061	0	0.014	0.948	0.016	2.463
10	DP-5	0	0.62	0	0.395	0.032	0.65	0.073	0.046	1.816
11	DP-6	0.002	0	0.255	0	0.002	0.98	0	0.031	1.27
12	DP-7	0	0.43	0.915	0.022	0	0.059	0.132	0.035	1.593
13	NS-1	0	0	1.345	0	0	0.649	0.367	0	2.361
14	NS-2	0	0.426	1.415	0.041	0.86	0	0.418	0	3.16
15	NS-3	0	0.929	0.981	0	0.222	0	0.652	0.085	2.869
16	NS-4	0	0.89	0	0.031	0.09	0.023	0	0	1.034
17	NS-5	0.436	0.138	0	0.051	0	0.32	0.47	0	1.415
18	NS-6	0.042	0.16	0.19	0	0	1.52	0.4	0.52	2.832
19	NS-7	0.065	0	0.47	0	0	0.078	0	0.485	1.098
	Mean	0.038	0.358	0.555	0.032	0.130	0.486	0.426	0.065	2.091
	STD	0.099	0.342	0.565	0.090	0.226	0.554	0.342	0.156	0.975
	95% confid.	0.001	0.005	0.008	0.001	0.003	0.008	0.005	0.002	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, SK: Sakrund, DP: Daulatpur, NS: Nawabshah.

0.059 mg kg⁻¹, 0.132 mg kg⁻¹, 0.132 mg kg⁻¹ and 0.035 mg kg⁻¹ respectively.

In soil sample NS-1 the residues of mevinphos, dimethoate, methyl parathion, chlorpyrifos, endosulfan and profenofos were found in the quantity of 0.56 mg kg⁻¹, 1.345 mg kg⁻¹, 0.054 mg kg⁻¹, 0.649 mg kg⁻¹, 0.367 mg kg⁻¹ and 0.71 mg kg⁻¹, respectively. In soil sample NS-2 the residues of mevinphos, dimethoate, methyl parathion, fenitrothion, chlorpyrifos, endosulfan and profenofos were found in the quantity of 0.426 mg kg⁻¹, 1.415 mg kg⁻¹, 0.0415 mg kg⁻¹, 0.86 mg kg⁻¹, 0.59 mg kg⁻¹, 0.418 mg kg⁻¹ and 0.002 mg kg⁻¹, respectively. In soil sample NS-3 the residues of mevinphos, dimethoate, methyl parathion, fenitrothion, chlorpyrifos, endosulfan and profenofos were found in the quantity of 0.92 mg kg⁻¹, 0.981 mg kg⁻¹, 0.063 mg kg⁻¹, 0.225 mg kg⁻¹, 0.849 mg kg⁻¹, 0.652 mg kg⁻¹ and 0.085 mg kg⁻¹, respectively. In soil sample NS-4 the residues of mevinphos, methyl parathion, fenitrothion, chlorpyrifos, endosulfan and profenofos were found in the quantity of 0.89 mg kg⁻¹, 0.031 mg kg⁻¹, 0.09 mg kg⁻¹, 0.023 mg kg⁻¹, 0.94 mg kg⁻¹ and 0.319

mg kg⁻¹, respectively. In soil sample NS-5 the residues of dichlorvos, mevinphos, methyl parathion, chlorpyrifos, endosulfan and profenofos were found in the quantity of 0.436 mg kg⁻¹, 0.138 mg kg⁻¹, 0.051 mg kg⁻¹, 0.32 mg kg⁻¹, 0.47 mg kg⁻¹ and 0.014 mg kg⁻¹, respectively. In soil sample NS-6 the residues of dichlorvos, mevinphos, dimethoate, methyl parathion, chlorpyrifos, endosulfan and profenofos were found in the quantity of 0.042 mg kg⁻¹, 0.16 mg kg⁻¹, 0.192 mg kg⁻¹, 0.815 mg kg⁻¹, 1.52 mg kg⁻¹, 0.4 mg kg⁻¹, 0.52 mg kg⁻¹, respectively. In soil sample NS-7 the residues of dichlorvos, mevinphos, dimethoate, methyl parathion, fenitrothion, chlorpyrifos, endosulfan and profenofos were found in the quantity of 0.065 mg kg⁻¹, 0.06 mg kg⁻¹, 0.475 mg kg⁻¹, 0.745 mg kg⁻¹, 0.001 mg kg⁻¹, 0.078 mg kg⁻¹, 0.855 mg kg⁻¹ and 0.485 mg kg⁻¹ respectively.

Most of the soil samples collected from Nawabshah district were found contaminated with pesticide residues at different levels. The most widely detected pesticide was chlorpyrifos and found in 16 samples with mean concentration of 0.486 mg Kg⁻¹. Endosulfan was the second most

often detected pesticide investigated in 15 samples containing the mean concentration of 0.426 mg Kg^{-1} . Dimethoate was the third most detected pesticide in 14 samples with mean concentration of 0.555 mg Kg^{-1} . The mean of Σ pesticides was found to be $2.091 \pm 0.975 \text{ mg Kg}^{-1}$ at 95% confidence level in soil samples collected with highest value of 4.254 mg Kg^{-1} in sample SK-1 followed by 3.56 mg Kg^{-1} in SK-2, 2.882 mg Kg^{-1} in SK-4, 2.643 mg Kg^{-1} in DP-4, 2.361 mg Kg^{-1} in NS-1, 3.61 mg Kg^{-1} in NS-2 and 2.832 mg Kg^{-1} in sample NS-6 while, other samples (53%) were found contaminated at concentration less than 2 mg Kg^{-1} .

Presently the pesticides used are mostly synthetic organic compounds. The sediments may 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 ground / surface 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 district Nawabshah, Sindh. Most of the soil samples collected from these areas were contaminated with pesticides. The most widely detected pesticides which are currently or have been used heavily in the past included chlorpyrifos, endosulfan and dimethoate in 16%, 14% and 15% samples of soil from Nawabshah. On the other hand Anwar (2009) reported that in Bahawalpur, Punjab the mevinphos was detected in eleven samples while endosulfan and fenitrothion were detected in ten and seven samples respectively. Endosulfan and fenitrothion were found in all soil samples collected from Lodhran, Punjab. While chlorpyrifos,

dimethoate and mevinphos were found in eight samples and dichlorvos was found in four samples. 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 ($0.01 \mu\text{g L}^{-1}$) (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). These findings are in complete agreement with the present findings where endosulfan was detected in 15 out of 19 samples collected from Nawabshah district of Sindh, the cotton growing areas of Pakistan. Endosulfan due to its persistency and commonly used feature on crop was frequently found when applied in the past in the sediments.

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 and stay in the sediment, decomposed very slowly and may persist for several years as they are insoluble in water and are retained by the soils. DDT (long banned insecticide) 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). The detection of pesticides in soil and their findings are in full agreement to the present results of present study where the endosulfan, dimethoate and chlorpyrifos were detected in most of the samples. Dimethoate was found in 14 samples out of 19 from Nawabshah (present studies) while in six and eight samples it was detected out of twelve samples collected from Bahawalpur and Lodhran (Anwar, 2009) followed by profenofos in nine out of 19, two out of twelve and eight out of twelve samples collected from the

same areas respectively. 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 30 cm depth and DDT and its metabolites were detected at 90 cm depth in traces. These results are in complete agreement with the present results where the soil collected from three locations of cotton growing areas were found contaminated with dimethoate and profenofos in most of the collected samples. 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 ($<1 \text{ mg L}^{-1}$) 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 in 16 samples out 19 from Nawabshah district (present study) while it was detected only in one and eight samples of Bahawalpur and Lodhran respectively out of twelve soil samples (Anwar, 2009). 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. It is concluded that to prevent adverse effects on public health it is a must to carryout regular monitoring system of these kinds

for the establishment of safety measures.

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