Journal of Science and Sustainable Development (JSSD), 2013, 1(2) 25-40
Copyright © Ambo University
ISSN: 2304-2702 (print); 2414-4479 (online)
DOI: https://doi.org/10.20372/au.jssd.1.2.2013.015

GC-MS Determination of Pesticide Residues in Effluent Run-off, River and Adjacent Soil around a Floriculture Industry in Holetta Town, Ethiopia

[1]Attah, L. E. and [2]Melkamu Biyana

[1]*Department of Chemical Sciences, Cross River University of Technology, Calabar, Nigeria*
[2]*Department of Chemistry, Wollega University P. O.Box 395, Nekemte, Ethiopia*

Abstract

A variety of organophosphorous and organochlorine pesticides residues have been assessed in the effluent, river and adjacent soil around a floriculture industry in Holetta Town West Shoa Zone, Ethiopia. Extraction of the pesticides was carried out by solid-phase extraction (SPE), and quantification was carried out by Gas Chromatography-Mass Spectrometry (GC-MS). Pesticides recoveries ranged from 80-101% in water and 64-99.9% in soil samples after 1 week aging. The mean concentrations of dominant pesticides (carbofuran, propyzamide, oxamyl, chlorothalonil, endosulfan(α) and kerosoxim-methyl) follow the sequence: effluent>soil>river , ranging from 0.268 - 4.655 mg/L in the effluent, 0.066 - 2.800 mg/kg in the soil and 0.043 - 1.191 mg/L in the river sample except for malathion, fludioxonil, and p,p'-DDT which had higher concentrations in river than soil. Pesticide concentrations in all samples were above the maximum residue limit (MRL) set by EU for total pesticides concentration. The results of this study suggested that both the river and adjacent soil have been contaminated with the pesticides from the floriculture effluent run-off, which could result in adverse biological effect on the ecosystem.

Key words: Pesticides, Effluents, GC-MS, Solid phase extraction, Floriculture.

Introduction

Intensive agricultural practices often include the use of pesticides to enhance crop yield. However, the improvement in yield is sometimes concomitant with the occurrence and persistence of pesticides residues in soil and water (Ware and Whitacre, 2004). Pesticides may reach the soil through direct application to the soil surface, incorporation in the top few inches of soil or during application to crops (McEwen and Stephenson, 1979). Pesticides can enter ground water resources and surface run-off during rainfall, thereby contributing to the risk of environmental contamination. The fate of pesticides in soil and water environment is influenced by the physico-chemical properties of the pesticides, the properties of the soil and water systems, climate, biology and other factors (Singh, 2001).

Organ-chlorine pesticide (OCP) residues particularly the oxidized form of heptachlor, remain in soils in South Korea even though their use had been discontinued since 1980 (Kim and Smith, 2001). Residues of toxaphene, DDT, triflurain and hexachlorocyclohexane (lindane) have been detected in soils from cotton growing field in South Carolina (Kannan et al., 2003). Carvalho et al. (2002) identified chlorinated hydrocarbons and organophos-phorous pesticides in coastal lagoons of Nicaragua. The DDT's were the main contaminants detected in soils and water from Bajul and Dakar in West Africa (Manirakiza et al., 2003). A study of surface water quality in Ivory Coast demonstrated the occurrence of organochlorine pesticides at low concentrations (Wandan and Zabik, 1996). Niala et al. (1995) had reported that flower producers in Ecuador, use several types of pesticides annually. Several studies have been reported on the determination of residual pesticides levels in river and soil using GC-MS (Nakamura et al., 2001, Vega et al., 2005; Markovic et al., 2010; Bhupander et al., 2011; Heela-Kimhampa., 2011; Leena et al., 2012; Akan et al., 2013).

In the past decade, the floriculture industry has flourished in Ethiopia due to geographical advantage, availability of unexploited arable land, favorable climate for flower growing and strong incentive from Ethiopian government to encourage investment and growth of the industry as the second largest foreign exchange earner after coffee (Obole, 2009). The floriculture industries in Ethiopia use more than 300 chemicals including pesticides, insecticides, fungicides, nematodes and growth regulators as of the recent report by the Ministry of Agriculture of Ethiopia, Crop Protection Department, and Quarantine Office. Serious deterioration of the environment has been observed in Kenya where floriculture has prospered and is more advanced than Ethiopia (Silver and Riley, 2001; Obole, 2009).

Though the use of pesticides have increased in Ethiopia, little information is available regarding the environmental impacts resulting from their use (Obole, 2009) and very few information is known on the specific concentrations and types of pesticides in Ethiopian soil and water within the industrial establishments.

In this study, pesticides residues in floriculture effluent, river and adjacent soil were assessed to obtain information on the status of pesticide residues within the environment of floriculture industries in one of the fastest flower growing town, Holeta in Oromia Special Zone, Ethiopia.

Materials and Methods

Study area

The two floriculture greenhouses used in this study are located at Holetta town in Wadera district, which is 35 km, west of Addis Ababa, the capital city of Ethiopia.

Sampling sites

The floriculture greenhouses labeled F1 and F2, effluents sampling sites labeled E1, E2, E3 and E4, river bank water (R1 and R2) and adjacent soil (S1and S2) are shown in Fig 1.

Sampling

Effluent and river bank water samples were collected in sealed plastic bottles which have been thoroughly washed with detergent, rinsed with distilled water, soaked in 5% HNO_3 for about 24 hours and finally rinsed with doubly distilled water. Two samples were taken from the drain with effluent water from the floriculture enterprise (F1) at the outlet of the drain E1 and at the outflow to the river E4. Two samples were taken from the drain with effluent water from the floriculture enterprise (F2) at the outlet of the drain E2 and at the outflow to the river E3. River bank water sampless labelled R1 and R2 were collected in triplicates, few meters away from the points where the effluent drainage joins the river. The samples placed in an iced-chest containing ice and transported in an ice box to Ambo university chemistry laboratory where they were stored at 4^oC until analysis. Samples were analyzed within 24hrs of their arrival.

Soil samples labeled S1 and S2 were collected from 150m away from the floriculture greenhouses using a stainless steel plated Auger soil probe at depth ranging from top soil up to 25cm. For each soil sample, twelve composite soil samples were obtained in zigzag manner from approximately two hectares for each sample (S1 and S2)., manually mixed, air dried, ground in a mortar and pestle and sieved through a 75 μm pore sieve.

After this, the samples were wrapped with aluminum foil to avoid light aided decomposition of the pesticide and transported to Ambo University chemistry laboratory (AUCL) in an ice-packed container, and preserved at 4^oC in refrigerator until the time of use.

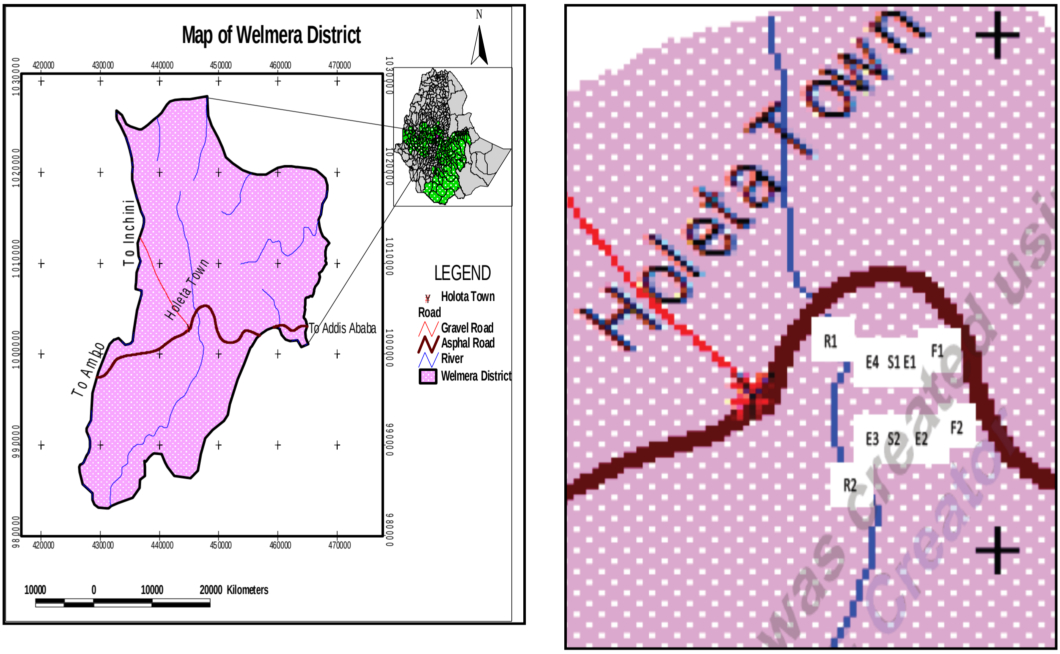

Figure1. Map of Welmera District showing Holetta Town and sample location sites

Pesticide standards and reagents

Pesticide standards such as Dichlorovos, Carbofuran, Profenofos, DDT and its isomers (o,p' DDT and p,p' DDT), Kerosoxim-methyl, Oxamyl, Malathion, Phorate, Propyzamide, Heptonophos, Endusulfan(α), Filuxilaxole, Chloro-thenlonil and Fludioxonil were purchased from Dr. Ehrendorfer (Ausburg, Germany). Anhydrous sodium sulfate, n-hexane, iso-octane (Nice chemicals Pvt. Ltd cochin-682024), methanol and ethyl acetate (UNICHEM, India) and HPLC grade acetonitrile (Fisher Scientific, England, UK) were used in this work. All solvents were of pesticide-residue grade and subjected to a solvent purity test for residue analysis suitability.

Extraction of pesticide residue

1L of effluent and river samples were first filtered separately using a Whatman filter paper (47μm). The pH was adjusted to 6.5 and the samples were extracted by solid phase extraction (SPE) using C18 cartridge (Bond Elute C18, Varian, USA) as described in the Pesticide Analytical Manual (USFDA, 1994). The sorbent was conditioned or wetted by consecutive additions of 5 mL of isooctane, ethyl acetate, methanol and 10 mL of double distilled water. 1L of the sample was loaded to the sorbent at a flow rate of 10 mL/min. under vacuum. The sorbent was rinsed using

10 mL of double distilled water and dried by aspirating air for 30 minutes. The analytes were eluted three consecutive times using 3×0.5mL of ethyl acetate followed by 3×0.5mL of isooctane.

Soil sample was extracted using the shake flask method in acetonitrile-water (70:30v/v) mixture as described in Kleiboher (2001). 25g of soil sample, 70mL of acetonitrile-water mixture were added in 250mL volumetric flask, capped and then shaken for one hour using mechanical shaker.

The supernatant was filtered using filter paper (Astme 832-81, 12.5cm, China). The extraction was repeated three times for a single sample with fresh solvent (20mL acetonitrile) as specified in the method.

Partitioning of the extract

Partitioning of the extracted samples was carried out according to the method of Association of Official Analytical Chemist (AOAC, 1999). The extracted sample was transferred into 250ml separatory flask and a mixture of 80mL n-hexane and 20mL of acetonitrile was added with vigorous shaking for 1min. After separation, the acetonitrile layer was collected and anhydrous sodium sulfate was added to remove any moisture. Another 20mL acetonitrile was added to the n hexane layer and the partitioning step repeated three times. Finally, the n-hexane portion was discarded while the acetonitrile portion was evaporated on a rotary evaporator to a volume of 10ml and ready for clean up using solid phase extraction (SPE) with C-18 bond cartridge.

Clean up of extract

The effluent, river and soil extracts were transferred separately into a glass chromatographic column containing bond elute C-18 cartridge which has been topped with a layer of anhydrous sodium sulphate. The column was first preconditioned by rinsing with iso-octane and the extracted sample was then transferred onto the column. The column was eluted with 200ml eluent (20% acetone and 80% hexane followed by a second elution of 100mL (50% ethyl acetate and 50% isooctane). The collected eluants were combined and then concentrated on a rotary evaporator and then dissolved in n-hexane to a volume of 10mL. An aliquot of each extract was transferred into a 2mL vial ready for analysis by GC-MS.

Analytical methods

Determination of residual concentration of pesticides

Pesticides concentration were determined by analysis of samples using a gas chromatography--mass spectrometry (Perkin-Elmer Clarus 600 model, USA) with selective ion detector (quadrupole) and a fused silica capillary incorporating a proprietary phase - 5% phenyl, 95% methylpolysiloxane (30 m x 0.32 mm i.d; film thickness 0.25 µm). The GC was operated under the following conditions: initial temperature 60°C, held for 5 min. and ramped to 280°C at 15 min, being held for 6min. The carrier gas (helium) was in constant flow mode at 1.0 mL/min. Splitless injection of a 1.0 µL volume was carried out at an injection temperature of 250°C with the purge valve on at 2 min. The mass spectrometer was operated in electron ionization (EI) mode with impact ionization voltage 70 eV, a transfer line temperature of 280°C, ion source 280°C, solvent delay 10.5min, full scan time of 0.2 sec, inter scan delay of 0.05 sec. and selected ion monitoring (SIM) mode.

Method validation

Validation of the method was done by calculating percentages recoveries of each standard pesticide. A spiking solution of 0.1, 1.0 and 3.0 mg/L containing 15 organochlorine and organphosphorous pesticides were prepared in hexane. Method blanks were determined by taking the blanks through the entire measurement process. Calibration standard curves were created and the pesticide residues were quantitatively determined by comparison with the standard solutions under the identical gas chromatography-mass spectrometry conditions. The reliability of analytical method was examined by analyzing the fortified and tested samples with known quantities of tested pesticides after extraction, clean up and analysis. Linearity range, mean recovery, and limit of determination (LOD) were determined.

Statistical analysis

All values of residual pesticides were obtained as mean (n = 5) ± standard deviation (SD). Data were subjected to one-way analysis of variance (ANOVA) to determine the differences in pesticides contents among different sample analysis.

Results and Discussion

Validation of the method

The OCPs were identified by their retention times and fragment ions which are listed in Table 1 along with data library of National Institute of Standards and Technology (NIST). The gas chromatogram for the mixture of pesticides standards is presented in Fig. 2. The efficiency of the extraction method used in this work was evaluated by means of recovery studies with the samples fortified at three different levels (Tables 2 and 3). The performance of the method was evaluated and linearity, recovery and limit of determination (LOD) were determined. A good linearity (r^2 >0.998) was found in the range 0.001-3.0µg/L for water and 0.001-3µg/kg for soil samples studied (RSD < 6.5 %.) Pesticides recoveries ranged from 80-101% in water and 63.4 -99.9% in soil samples (after 1 week aging). These values were within the normal acceptable ranges of 70 – 120 % (Åkerblom 1995) except for phorate which had a recovery value of 63.4%. Recovery values were higher in water samples than the soil samples for all compounds. This may be attributed to the interferences from organic matter and other compounds that are found more in the soil than the water samples. The blank reference samples had no traces of the targeted analytes, and thus confirming the practicability of the analytical protocols employed in the determination of the pesticide residues.

Table1: Main ions fragments of pesticides detected by MS

Pesticides	Main ion fragments (m/z)		
Dichlorovos	79	109	185
Heptenophos	89	109	124
Phorate	29	75	121
Carbofuran	122	149	164
Chlorothalonil	264	266	268
Malathion	93	125	173
Endosulfa(α)	195	231	241
Profenofos	97	337	339
Kerosoxim-methyl	117	132	207
Filusilazole	48	207	234
Fludioxonil	127	154	248
o,p’-DDT	165	235	237
p,p’-DDT	165	235	237

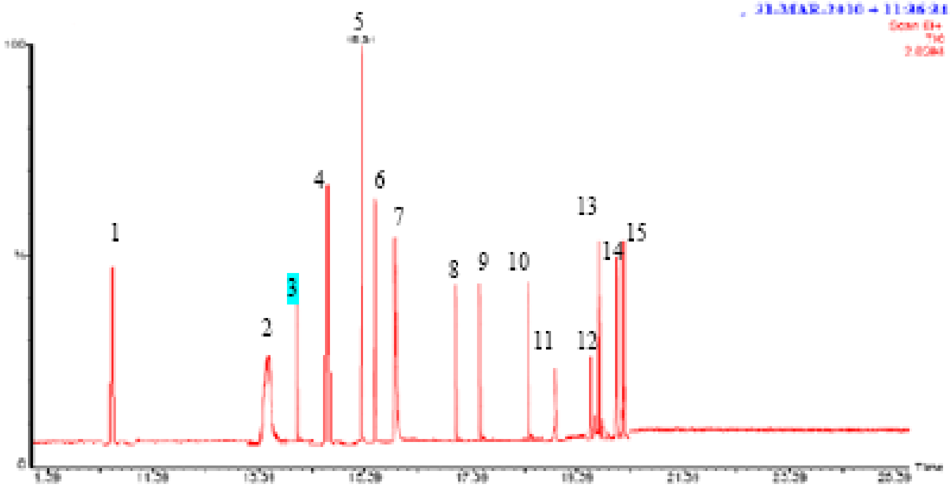

Figure.2: Gas chromatogram of pesticides standards mixture: 1. dichlorovos, 2. heptenofos, 3. phorate, 4. carbofuran, 5. propyzmide, 6. oxamyl, 7. chlorothalonil, 8. malathion, 9: endosulfan (α), 10. profenofos, 11. kerosoxim-methyl, 12. filusilazole, 13. fludioxonil, 14. o,p’-DDT and 15. p,p’-DDT

Table 2: Percentage recoveries, LOD, Linear range and Regression coefficient (r^2) of OCPs in water sample

Pesticide	Spiked level (µg L^{-1})	Mean recovery (%)	RSD (%) n=5	LOD (µg L^{-1})	Linear range (µg L^{-1})	Regression coefficient (r^2)
Dichlorvos	0.1	95.4	3.8	0.005	0.1-3	0.991
	1	96.6	3.6			
	3	97.5	3.4			
Carbofuran	0.1	96.2	5.2	0.005	0.1-3	0.994
	1	97.5	5.3			
	3	99.0	5.6			
Profenofos	0.1	81.7	3.6	0.003	0.1-3	0.997
	1	82.5	4.2			
	3	84.5	4.2			
o,p'-DDT	0.1	93.5	5.5	0.001	0.1-3	0.998
	1	94.8	6.1			
	3	99.8	6.0			
p,p'-DDT	0.1	95.6	4.5	0.005	0.1-3	0.998
	1	96.5	5.8			
	3	101.0	4.2			
Krosoxim-methyl	0.1	82.7	4.2	0.001	0.1-3	0.995
	1	83.7	3.6			
	3	87.2	3.2			
Malathion	0.1	90.5	5.0	0.002	0.1-3	0.991
	1	92.9	4.3			
	3	96.1	3.8			
Phorate	0.1	82.5	3.	0.001	0.1-3	0.996
	1	84.8	2.9			
	3	87.3	4.1			
Heptenophos	0.1	88.9	4.2	0.005	0.1-3	0.998
	1	89.6	3.2			
	3	91.4	5.8			
Endosulfan(α-isomer)	0.1	96.5	5.9	0.005	0.1-3	0.994
	1	97.4	6.1			
	3	98.9	4.6			
Filusilazole	0.1	92.5	4.1	0.002	0.1-3	0.993
	1	93.2	3.6			
	3	95.1	3.2			
Chlorothalonil	0.1	94.5	5.9	0.002	0.1-3	0.999
	1	96.8	5.6			
	3	98.7	6.4			
Fludioxonil	0.1	75.2	6.1	0.001	0.1 -3	0.994
	1	78.6	4.6			
	3	80.8	5.2			

Table 3.Percentage recoveries, LOD, Linear range, RSD and regression coefficient (r^2) of OCPs in soil sample

Pesticides	Spike level (µg/kg)	Mean recovery (%)	RSD (µg/kg)	LOD (µg/kg)	Linear range (µg/kg)	Regression coefficient (r^2)
Dichlorvos	0.1	68.7	4.5	0.001	0.1-3	0.991
	1	68.4	5.2			
	3	79.3	5.2			
Carbofuran	0.1	106.3	5.1	0.003	0.1-3	0.994
	1	96.6	4.5			
	3	87.3	3.4			
Profenofos	0.1	69.7	4.2	0.005	0.1-3	0.997
	1	78.4	3.2			
	3	73.9	3.6			
o,p'-DDT	0.1	72.4	4.2	0.001	0.1-3	0.998
	1	72.5	4.6			
	3	70.3	6.3			
p,p'-DDT	0.1	107.2	4.8	0.003	0.1-3	0.998
	1	101	3.8			
	3	87.9	4.5			
Krosoxim-methyl	0.1	95.0	3.2	0.003	0.1-3	0.995
	1	102	5.6			
	3	99.9	5.3			
Oxamyl	0.1	99.8	5.5	0.001	0.1-3	0.992
	1	99.3	3.8			
	3	94.9	3.1			
Malathion	0.1	97.5	4.4	0.005	0.1-3	0.991
	1	85.1	2.4			
	3	74.3	6.4			
Phorate	0.1	75	3.3	0.001	0.1-3	0.996
	1	68.7	5.6			
	3	63.4	4.4			
Propyzamide	0.1	88.1	3.6	0.003	0.1-3	0.998
	1	75.0	3.2			
	3	92.2	5.0			
Heptenophos	0.1	105	4.5	0.001	0.1-3	0.998
	1	81.5	4.1			
	3	80.7	3.6			
Endosulfan(α)	0.1	89.4	5.7	0.001	0.1-3	0.994
	1	71.9	6.1			
	3	68.9	5.4			
Filusilazole	0.1	95.4	3.5	0.005	0.1-3	0.993
	1	86.4	4.8			
	3	79.4	3.9			
Chlorothalonil	0.1	88.7	5.8	0.005	0.1-3	0.999
	1	94.5	3.5			
	3	96.8	2.4			
Fludioxonil	0.1	77.5	4.3	0.003	0.1-3	0.994
	1	85.3	6.3			
	3	82.4	4.1			

Determination of pesticides residue

The chromatogram of residual pesticides detected in effluent sample is presented in Fig. 3. The results of organochlorine and organophosphate pesticides analyzed in the effluent, river and soil samples are shown in Tables 4, 5 and 6.

In the effluent, the mean concentrations of pesticides varied from 0.016-4.376 (µg/L) in E1 with the highest concentration found in oxamyl (4.376 µg/L) while the lowest concentration was found in phorate (0.016.µg/L). Pesticides concentration in E2 ranged from 0.013-4.547(µg/L) with carbofuran recording the highest concentration (4.454µg/L) while malathion and phorate recorded the lowest concentration (0.013 µg/L). Mean concentration in E3 ranged from 0.022 -4.736 (µg/L). The highest concentration was found in carbofuran (4.736 µg/L) while the lowest concentration was found in profenos and heptanophos. 0.022 (µg/L). Mean concentration in E4 ranged from 0.008-4.684(µg/L) with carbofuran recording the highest concentration (4.684 µg/L) and profenofos, the lowest concentration (0.008 µg/L). Dichlorovos, phorate, and profenofos were found in small quantity in all the effluents, while kerosoxim-methyl, filusilazole, fludioxonil and o,p' DDT were not detected in effluents E2 and E4. 2,4D herbicide was detected in all the effluent samples.

Residual pesticides concentration in river sample ranged from 0.003-0.210 (µg/L) in R1, with the highest concentration found in carbofuran (0.210 µg/L) while the lowest concentration was found in heptanofos (0.003 µg/L). In R2, pesticide concentration ranged from 0.004-2.170 (µg/L) with the highest concentration found in carbofuran (2.170 µg/L) and the lowest found in heptanofos.(0.004µg/L). Phorate, oxamyl, endosulfan (α) and profenofos were found intrace levels in R1 while kerosoxim-methyl, filusilazole, fludioxonil and o,p' DDT were not detected in R1.

In the soil sample, pesticide concentrations ranged from 0.032-3.790 (µg/kg) in S1, with the highest concentration found in propyzamide (3.790 µg/kg) and the lowest found in phorate (0.032µg/kg) while in S2 the concentration ranged from 0.048-3.700 (µg/kg) with the highest concentration found in carbofuran (3.700 µg/kg) and the lowest found in dichlorovos.(0.048 µg/kg). Profenofos, filusilazole, and o,p' DDT were not detected in any of the soil samples. Phorate, propyzamide, chloro-nthalonin, endosulfan (α) and p,p' DDT were not detected in S1 while kerosoxim-methyl, fludioxalin, oxamyl and malathion were not detected in S2.

A comparison of mean concentrations of dominant pesticides in the effluent, river and soil samples is presented in Figure 4.

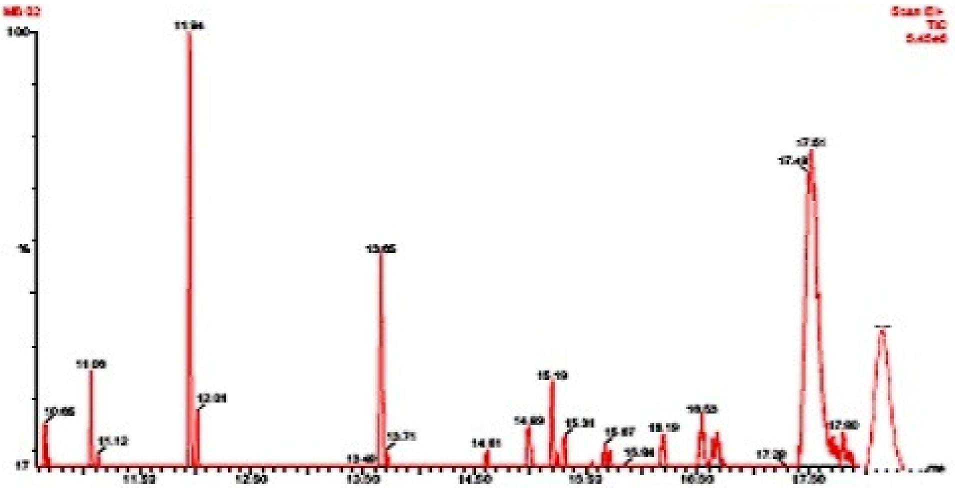

Figure 3: GC chromatogram of residual pesticides in effluent

Table 4: Concentrations of residual pesticides in effluents (µg/L)

Pesticide	Effluent samples			
	E1	E2	E3	E4
Dichlorovos	0.059±0.02	0.044±0.011	0.055±0.01	0.051±0.03
Heptenophos	0.158±0.001	0.040±0.001	0.024±0.01	0.011±0.001
Phorate	0.016±0.001	0.013±0.001	0.033±0.012	0.032±0.001
Carbofuran	4.657±0.01	4.547±0.12	4.736±0.02	4.684±0.06
Propyzamide	1.894±0.03	1.800±0.02	1.989±0.01	1.894±0.02
Oxamyl	4.736±0.01	2.131±0.04	0.237±0.03	0.218±0.01
Chlorothalonil	0.947±0.03	0.578±0.03	1.500±0.02	1.184±0.03
Malathion	0.026±0.01	0.013±0.001	0.757±0.01	0.276±0.01
Endosulfa(α)	2.273±0.21	1.061±0.02	0.303±0.003	0.258±0.01
Profenofos	0.021±0.001	0.019±0.001	0.022±0.001	0.008 ±0.001
Kerosoxim-methyl	0.302±0.01	ND	0.521±0.01	0.451±0.01
Filusilazole	0.042±0.001	ND	0.461±0.01	ND
Fludioxonil	0.122±0.01	ND	0.949±0.01	ND
o,p'-DDT	0.071±0.01	ND	0.595±0.02	ND
p,p'-DDT	1.211±0.01	0.795±0.01	1.932±0.02	0.118±0.01
2,4-D	0.512±0.01	0.273±0.001	0.374±0.01	0.132±0.02

Table 5. Concentrations of residual pesticides in Holetta River samples (µg/L)

Pesticide	River samples	
	R1	R2
Dichlorovos	0.008±0.002	0.044±0.001
Heptenophos	0.003±0.001	0.008±0.002
Phorate	0.004±0.002	0.024±0.001
Carbofuran	0.210±0.01	2.170±0.01
Propyzamide	0.060±0.01	0.473±0.02
Oxamyl	0.020±0.001	1.730±0.001
Chlorothalonil	0.080±0.02	0.947±0.02
Malathion	0.084±0.01	0.268±0.01
Endosulfa(α)	0.006±0.001	0.378±0.002
Profenofos	0.010±0.001	0.011±0.001
Kerosoxim-methyl	ND	0.087±0.01
Filusilazole	ND	0.421±0.001
Fludioxonil	ND	0.884±0.01
o,p'-DDT	ND	0.074±0.001
p,p'-DDT	0.032±0.01	1.491±0.02

Table 6. Concentration of pesticides in soil samples (µg/kg).

Pesticides	Soil samples	
	S1	S2
Dichlorovos	0.047±0.011	0.048±0.012
Heptenophos	0.048±0.001	0.051±0.001
Phorate	0.032±0.001	ND
Carbofuran	1.890±0.01	3.700±0.001
Propyzamide	3.790±0.002	ND
Oxamyl	ND	2.710±0.003
Chlorothalonil	1.180±0.02	ND
Malathion	ND	0.012±0.001
Endosulfa(α)	0.416±0.01	ND
Profenofos	ND	ND
Kerosoxim-methyl	ND	0.391±0.001
Filusilazole	ND	ND
Fludioxonil	ND	0.176±0.01
o,p'-DDT	ND	ND
p,p'-DDT	0.098±0.002	ND

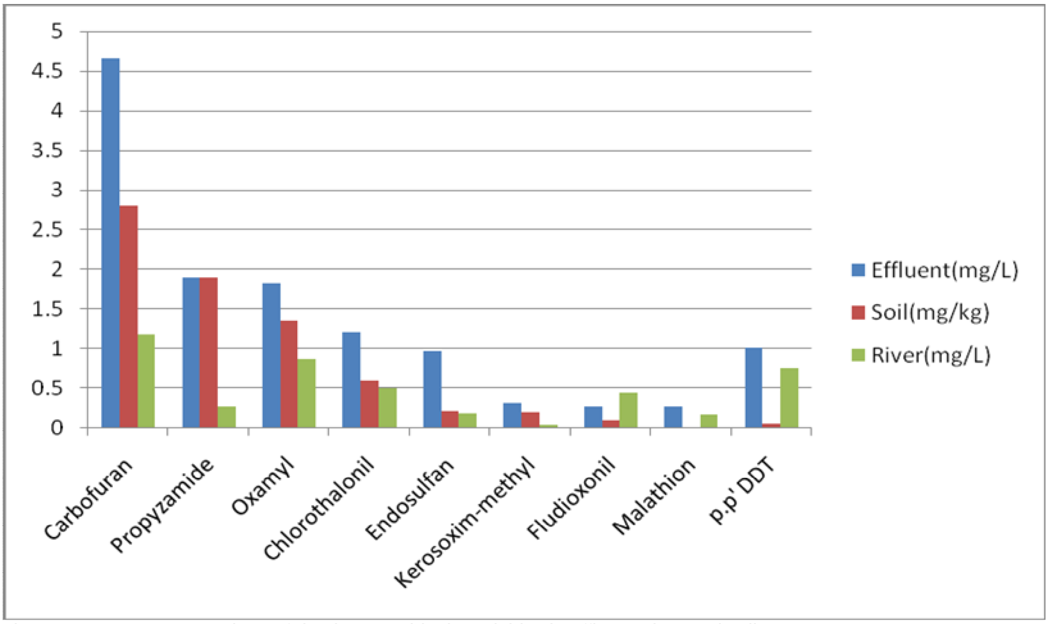

Figure 4: Mean concentrations of dominant residual pesticides in effluent, river and soil

Discussion

Residual pesticides detected in the effluent

High concentrations of organochlorine pesticides (carbofuran, propyzamide, oxamyl, chlorothalonil, p,p'DDT, and endosulfan (α)) were detected in all effluent samples with carbofuran being the most dominant pesticide with a mean concentration of 4.655 µg/L. A high concentration of kerosoxim-methyl (0.323 μg/L) was detected in 75% of the samples while significant amount of malathion and fludioxonil were detected in 50% of the effluent (Table 3) Organophophosphorous pesticides (dichlorovos, heptanofos, profenofos and phosphate) were detected in small quantities in the three samples studied.

The dominant pesticides are strong toxicants and their direct discharge from the floriculture greenhouse can generally affect the health of the environment. Endosulfan is a highly toxic pesticide in Environmental Protection Authority (EPA) toxicity. The solubility of endosulfan is 0.3 mgl^{-1} with a half-life of 5 weeks in water. DDT is a persistent organic pollutant which is nearly insoluble in water with a half-life of 2–15 years. The herbicide 2,4-D was also detected in all effluent samples at relatively high concentrations, but the level was below the maximum residual level (MRL) of 10mg/L established by EU. Although the concentration of residual pesticides in this work were considerably high but no (MRL) for these pesticides in horticulture effluent have been established.

The presence of residues of pesticides in the effluent analyzed can be attributed to the excessive application of pesticides in the floriculture industry. Persistent organochlorine pesticides such as DDT and endosulfan have been used by flower industries in Mexico, while dichlorovos, endosulfan, malathion and chlorothalonil have been used by Colombian rose greenhouses (Niala et al., 1995) and their high concentrations in the floriculture effluent in this work which agreed with that reported by Leena et al (2012) may be attributed to the application of these pesticide in the floriculture industry under study.

Residual pesticides detected in river samples

The analysis showed that all the pesticides studied were detected in R2 while 75% occurrence was detected in R1 (Table 4). Organochlorine pesticides (carbofuran, propyzamide, oxamyl, chlorothalonil, endosulfan and p,p' DDT were higher in R2 than R1. The dominant pesticides carbofuran, oxamyl propyzamide, oxamyl, chlorothalonil, endosulfan and p,p' DDT with mean concentrations of 1.191mg/L, 0.875 mg/L, 0.266 mg/L, 0.50 mg/L, 0.192 mg/L and 0.762 mg/L respectively were higher than those reported in other studies (Papadopoulou-Mourkidou et al 2004; Ukpadhi and Wokoma,2012; Leena et al 2012) and above the MRL set by European Union (EU) for the protection of human health as no standards are available for Ethiopia. The difference in concentrations of residual pesticides in R1 and R2 may be due to the flow of effluents carrying pesticides and other chemicals from the floricultural industry into the river. Hence, the effluents released from the industry are the primarily responsible for the increase in concentration of pesticides in R2.

Pesticides concentration in water though at relatively low concentrateions is relatively common in areas of intensive farming or near hazardous waste disposal sites. Of the pesticides detected in water, malathion with mean concentration of 0.176 mg/L is considered highly toxic to fish and other aquatic organisms, while others are considered moderately toxic (Leena et al, 2012) Endosulfan is a highly toxic pesticide in EPA toxicity ranking and its solubility is 0.3 mg/L with a DT50 of 5 weeks in water. Lower concentrations of pesticides in river water in this work, which agreed with that reported by Asi (2003) and Heela-Kihampa (2011), may be attributed to the hydrophobic nature of pesticides that make their presence in water in small quantities. Although the concentrations of the pesticides in river sample found in this work were low, they exceeded the MRL set by EU, suggesting that the river water was contaminated by the pesticides residue.

Residual pesticides detected in soil samples

Dichlorovs, heptenofos, and carbofuran were identified in all of the soil samples while the rest of the pesticides analyzed in this work were found only in 50% of the samples. The dominant pesticides, carbofuran (2.80 μg/kg), propyzamide (1.895 μg/kg), oxamyl (1.355 μg/kg)), chlorathanolin (0.59 μg/kg), endosulfan (0.208μg/kg) and kerosoxime-methyl (0.195 μg/kg) found in this work though lower than those reported in other studies for agricultural soil (Sanchez-Brunete et al, 2004; Vega et al 2005, Ramasamy and Vasudevan, 2006; Bhupander et al. 2011; Aiyesanmi and Idowu, 2012) were above the MRL set by EU. p, p' DDT was found in 50% of the soil sample and is immobile in most soils but the concentration detected was lower than the reported value by Akan et al (2013). The level of residual pesticides in the soil can be attributed to both the effluent run-off, the contaminated river water used for irrigation of the adjacent farmland and excessive use of pesticides in the agricultural farmland.

Comparison of dominant residual pesticides

The mean concentration of most of the pesticides residue levels follow the sequence; effluent>soil>river. The dominant organochlorine pesticides, carbofuran, propyzamide, oxamyl, chlorathanolin, endosulfan, and kerosoxim-methyl were higher in the effluent and soil than in river water except fludioxonil, malathion and p,p'DDT, which had higher levels in river than in the soil (Figure 4). 2, 4 D was found only in the effluent but was not detected in both the river and soil samples. Organophosphate pesticides (dichlorovos, heptanophos, profenofos and phosphate) detected in all the samples were much lower than that reported by Akan et al. (2013).

Conclusions

The results obtained in this study revealed that there exist a variety of organophosphorous and organochlorine pesticides residues in the effluent run-off from the horticulture industry, river water and adjacent soil. The dominant organochlorine pesticides, carbofuran, propyzamide, oxamyl, chlorathanolin, endosulfan(α) and kerosoxim-methyl were higher in the effluent and soil than in river water except fludioxonil, malathion and p,p'DDT which had higher levels in river than in the soil. The concentrations of pesticides in the effluent waters from the floricuture enterprise at Holetta were generally above the thresholds where ecotoxicological effects on organisms can be expected. Discharge of concntrated effluent water to the river, may therefore have adverse impact on the local aquatic ecosystem.

A holistic approach on the assessment of environmental impact of continuous use of pesticides by the flower industry should be adopted with adequate monitoring and appropriate legislation be enacted in order to check the excesses by this enterprise.

Acknowledgements

The authors wish to acknowledge both Wollega University and Ambo University Research and publication office for the material and financial support for this study.

References

Aiyeanmi, A. P. and Idowu, G. A. 2012. Organchlorine pesticides residues in soil of cocoa farms in Ondo state Central district, Nigeria. Environment and Natural resources Research. Vol.2.No.2: 25-35.

Akan, J. C; Jafiya, L; Mohammed, G. and Abdulrahman, F. I 2013. Organophosphorus pesticide residues in vegetables and soil samples from alau dam and gongulong agricultural areas, Borno State, Nigeria. International Journal of Environmental Monitoring and Analysis. 1(2): 58-64.

Åkerblom, M. 1995. Guidelines for environmental monitoring of pesticide residues for the SADC region. SADC/ ELMS, Monitoring Techniques series 3 Lesotho, pp254.

AOAC International. 1999. Official methods of analysis of AOAC International, 16th ed.; Cunnif P, Ed; Gaithersburg, MD.

Asi, M. A. 2003. Solid-phase extraction and chromatographic determination of pesticides in food and water samples, Unpublished MSc thesis submitted to the University of the Punjab, Pakistan.

Bhupander, K; Richa, G; Gargi, G; Meanu, M; Kumar, S.S; Dev, K and Sanjay, K. 2011. Residues of pesticides and herbicides in soils from agriculture areas of Delhi region, India. Journal of Environment and Earth Science. vol.1(2):21-30.

Carvallo, F. P; Villeneuve, J. P; Cattini, C; Tolosa, I; Montenegro-Guillen, S; Lacayo, M and Cruz, A. 2002. Ecological risk assessment of pesticide residues in coastal lagoons of Nicaragua. J. Environ. Monit. 4:778-787.

EU council directive, 98/83 EC of 3 Nov. 1998 on the quality of water intended for human consumption. Off. J. of the Eur. Comm, L. 330:32-54.

Heela-Kihampa, H. 2011. Pesticides residues in four rivers running through an intensive agricultural area, Kilimanjaro, Tanzania. J. Appl. Sci. Environ. Manage. vol.15 (2): 307-316.

Kannan, K; Battula, S; Loganathan, B. G; Hong, C.S; Villeneuve, D. L; Sajwan, K; Giesy,. J. P and Aldous, K. M. 2003. Trace organic contaminants, including toxaphene and trifulalin, in cotton field soils from Georgia and South Carolina, USA. Arch. Environ.Contam.Toxicol. 45:30-36.

Kim, J. H. and Smith, A. 2001. Distribution of organochlorine pesticides in soils from South Korea. Chemosphere 43: 137-140.

Kleibohmer, W. 2001. Handbook of analytical separations, Vol. 3, Elsevier, Amsterdam-London-New York-Oxford-Paris-Shannon-Tokyo, 163-182.

Leena, S; Chouldhany, S. K and Singh, P. K. 2012. Residue concentration in water and sediments of River Ganga at selected sites in middle Ganga plane. International Journal of Environmental Sciences vol.3, No 1, 260-274.

Manirakiza, P; Akinbamijo, O; Covac, A; Pitonzo, R and Scheps, P. 2003. Archieves of Environmental Contamination and Toxicology 44:171.

Markovic, M; Cupac, S; Durovic, R; Millerovic, J. and Klioji, P. 2010. Assessment of heavy metals and pesticides levels in soil and plant products from agricultural area of Belgrade, Serbia. Arch.Envron Contam Toxicol. 58(2):341-351.

McEwen, F. L. and Stephenson, G.R. 1979. The Use and Significance of Pesticides in the Environment. John Wiley & Sons, New York. Pp. 538.

Nakamura, S; Yamagani, T and Daishima, S. 2001. Determination of organochorine pesticides in river water by gas chromatography-negative ion chemical ionization mass spectrometry using large volume injection. Analyst, 126:1658-1662.

Niala, M; Dorren, M. and Gaston, T. 1995. The Game of the Rose. The Netherlands; International Books. 37-61.

Obole, T. L. 2009. Ethiopia: Cut Flower Bonanza: At what cost? JimmaTimes, 1-7.

Papadopoulou-Mourkidou, E; Karponzoa, D. G; Pasias, J; Kotopoulou, A; Mitothridon, A; Kintzikoglou, K and Vlachou, P. 2004. The potential of pesticides to contaminate the ground water resources of the Axios river basin PartII. Monitoring study in the south part of the river basin. Sci Total Environ, 321 (1-3):147-164.

Ramasamy, J. and Vasudevan, N. 2006. Residues of organochlorine pesticides in agricultural soils of Thiruvallur district, India. Food, Agriculture and Environment.vol4. Issue1, 313-316.

Sanchez-Brunete, C; Alberto, B. and Tadeo, J. L. 2004. Multiresidue determination of pesticides in soil by gas-chromatography-mass-spectrometry detection. Journal of Agricultural and Food Chemistry. 52 (6):1445-1451.

Silver, J and Riley, B. 2001. Environmental Impact of pesticides Commonly Used on Urban Landscapes, NCAP, and Eugene, Oregon, USA, 1-13.

Singh, R. P. 2001. Comparison of organo-chlorine pesticide levels in soil and groundwater of Agra, India. Bull.Environ. Contam.Toxicol. 67:126-132.

Tadeo, J. L. 2008. Analysis of pesticides in food and environmental samples, CRC Press, Taylor & Francis Group, Milton Park.pp220.

Upadhi, F and Wokoma, O. A. F. 2012. Examination of some pesticides residues in surface water, sediment and fish tissue of Elechi creek, Niger Delta, Nigeria. Research Journal of Environment and Earth Sciences, 4(11): 939-944.

USFDA. 1994. Pesticides analytical manual, volume 1, Multiresidue methods, 3rd edition. Available at http://v.cfsan.fda.gov/frf/pami3.htm (retrieved April, 2010).

Vega, A. B; Frenich, A. G. and Vidal-Martinez, J. L. 2005. Monitoring of pesticides in agricultural water and soil samples from Andalusi by liquid chromatography coupled to mass spectrometry. Analytica Chimica Acta. 538:117-127.

Wandan, E. N and Zabik, M. J. 1996. Assessment of surface water quality in Cote d'Ivoire. Bull.Environ. Contam. Toxicol. 56:73-79.

Ware, G. W and Whitaker, D. M. 2004. The Pesticide Book, MeisterPro Information Resources, Willoughby, Ohio. Pp. 496.