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Determination of Organophosphorus Pesticide Residues in Fodder Samples along Musi River Belt, Hyderabad, India

Kotinagu Korrapati[1*], Karthik Kotha[2] and Krishnaiah Nelapati[3]

[1]*Department of Veterinary Public health and Epidemiology, College of veterinary Science, Rajendranagar, Hyderabad, Telangana, India*
[2]*Department of Livestock products technology, College of veterinary Science, P.V. Narsimharao Veterinary University, Rajendranagar, Hyderabad, Telangana, India*
[3]*Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, P.V. Narsimharao Veterinary University, Rajendranagar, Hyderabad, Telangana, India*

**Corresponding author*

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A B S T R A C T

The present study was conducted to find the organophosphorus pesticide (OPP) residues in fodder samples along Musi river belt, India. Fodder samples collected from the six zones of Musi river belt, Hyderabad India were analyzed by gas chromatography with electron capture detector and pulsated flame photometric detector for the presence of OPP residues. The gas chromatographic analysis of fodder samples of Zone II, III, IV and VI of Musi river showed the residues of phorate at concentration of 0.42 ± 0.001 (0.421-0.428), 0.05 ± 0.02 (0.026-0.101), 0.27 ± 0.23 (0.01-0.561) and 0.36 ± 0.01 (0.369-0.385) ppm respectively. The residues of Dichlorovas, Quinolphos, Methyl parathion, Fenitrotion, Profenphos, Ethion, Phosalone, Lambda Cyhalotrin were below the detection limit in the remaining fodder samples collected from Musi river belt in the present study.

Introduction

Pesticide residues mean any specified substances in food, agricultural commodities or animal feed resulting from use of pesticides. The term includes any derivatives of pesticides such as conversion products, metabolites, reaction products and impurities considered being of toxicological significance (Codex Alimentarius Commission, 1993). The prolonged usage of persistent and harmful pesticides have raised a number of concerns, over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non-target species, air, water, bottom sediments and food.

The pesticides spread into the environment and has detrimental effect on human health through the contamination of soil, air and water resources and poses significant risks to the environment and non-target organisms, such as beneficial species of insects, soil, micro-organisms, plants and bird and cause development of insect resistance and resurgence of resistance in certain species of pests (Miller, 2004). There are several classes of pesticides including insecticides (protect against and/or control insect infestations) fungicides (protect against and/or control the spread of fungal diseases), molluscicides (control the destructive effects of slugs and snails) and rodenticides (control the activities of rats and mice). Insecticides used in the agricultural activities can be classified into 4 major groups as-organochlorines, organophosphates, carbamates and synthetic pyrethroids (Jeyaratnam, 1985).

Organophosphorous pesticides, less persistent than OCs, are frequently preferred choice of treatment because they are efficacious, bio-degradable and cost effective in control of wide range of pests. However, they are generally more toxic than OCs as potent cholinesterase inhibitors or carbamate compounds (Moye, 1981).

In India, 51% of food commodities are contaminated with pesticide residues and out of these, 20% have pesticide residues above the maximum residue level on a worldwide basis (Gupta, 2004). India, being a tropical country, the consumption pattern of pesticides is skewed towards insecticides, which account for 52% of total chemical pesticide consumption, herbicides 33% and fungicides 15% (Singhal, 2003). Almost 67% of the pesticides manufactured in India are used for 2 crops; namely rice and cotton, which are grown from July to November (Anonymous, 1996).

India is the 2nd largest manufacturer of basic pesticide chemicals in Asia, next to China (Hundal *et al.*, 2006), 13th largest exporter of pesticides in the world (Prabhu, 1999). The average pesticide consumption in India (480 g/ha/annum) is quite low against 17 kg/ha/annum in Taiwan, 10.70 kg/ha/annum in Japan, 3.5-4.5 kg/ha/annum in USA and 2.5-3.5 kg/ha/annum in Europe (Madan, 2006; Mayee, 2007). Among various Indian states, Andhra Pradesh had the highest consumption of pesticides (20%), followed by Punjab (10%) and Tamilnadu (9%) (Ramesh and Balasubramanian, 1999).

Animal feed containing pesticides is the main source for accumulation of these residues in tissues of animals (Gill *et al.,* 2001), which is directly proportional to their concentration in feed (Sharma *et. al.*, 1984) although inhalation of polluted air and absorption through intact skin also may take place. During lean season, when fodder is not available for animals, exposure of pesticides occurred through ingestion of soil from pasture land (Buck, 1973). As their concentration exceeds a certain threshold level, pesticides are translocated to mammary glands and are secreted in milk as residues (Anonymous, 1996). No segment of population is completely protected against exposure to pesticides and the potentially serious health effects leading to pesticide poisoning in about 1 million cases per year (Anonymous, 1999), though a disproportionate burden is shouldered by the people of developing countries and by high risk groups in each country (WHO, 1990).

Musi River is located on the Deccan platue in the state of Telangana, India. However, now the water is highly polluted as 600 million liters per day of untreated sewage water is discharged into Musi River, additionally 14 industrial estates drain their untreated effluents into this river. The agricultural drained water is another source of pollution and this river water is rich in heavy metals, pesticide residues, phenols, oils, grease, alkalis and acids (Cheepi, 2013). The self-purifying property of river water is unable to clear the pollution, and the polluted water poses a serious risk to public health especially in areas where river water is used for irrigation.

Keeping this in view of the Musi river pollution and its direct or indirect effect on environment, animal and human system, a study was conducted to analyze the fodder samples on the banks of river Musi for the presence of pesticide residues. The study has been conducted on river Musi, Located in Telangana, India.

Materials and Methods

Collection of samples

This study was based on 48 fodder samples collected from six divided zones (8 from each zone) (Table 1) (Figure 1) on the downstream of Musi river belt, Telangana, India in 2013. Zones were divided based on earlier reports on Musi river pollution by Cheepi, 2013. Approximately, 250 g of fodder samples were collected in sterilized polyethylene packs, packed and transported to lab. Samples were subjected to analysis within 24 h from their arrival.

Pesticides analyzed

The residues of certain pesticides of organophosphates (Dichlorovas, Phorate, Methyl parathion, Fenitrotion, Quinolphos, Profenphos, Ethion, Phosalone, Lambda Cyhalotrin) in fodder samples collected from six zones of Musi river belt area.

Chemicals and reagents

Acetonitrile, acetone, dichloromethane, graphitized carbon black, hexane, magnesium sulfate, silica gel, sodium chloride, sodium sulfate, prostate specific antigen (PSA) of high-performance liquid chromatography residue grade obtained from Qualigens and Merck specialties Pvt. Ltd. Analytical standards with >99% purity were obtained from Dr. Ehrenstorfer, Germany during 2012 and stored in deep free maintained at −40°C.

Method validation

The required quantity of (Organophosphorus) international standards prepared from certified reference materials were added to each 15 g sample to get fortification levels of 0.05 ppm and 0.1 ppm in three replications each. The AOAC official method 2007.01 with slight modifications was validated for the estimation of the limit of quantification (LOQ) of organophosphorus in fodder. Fodder samples were chopped, and 7.5 g of sample was taken into 50 ml centrifuge tubes and 30 ml of acetonitrile was added and shaken well. The sample was homogenized at 14000-15000 RPM for 2-3 min using heidolp silent crusher then 3 g of sodium chloride was added, mixed well by shaking gently then it was centrifuged at 2500-3000 RPM for 3 min to separate the organic layer, approx. 16 ml of organic layer was taken into a test tube and 9 g of anhydrous sodium sulfate was added to remove moisture (Kampire *et al.,* 2011). Taken about 0.4 g PSA sorbent and 1.2 g anhydrous magnesium sulfate into 15 ml centrifuge tubes. The 8 ml of organic layer extract was transferred into this 15 ml centrifuge tube, capped and vortex for 30 s, then tubes were centrifuged at 2500-3000 RPM for 5 min then 2 ml of extract was transferred into test tubes and the solvent (acetonitrile) was evaporated turbovap concentrator for GC analysis.

A Schimadzu (2010) gas chromatography (GC) equipment with a VF-1MS capillary column and with electron capture detector (ECD) and flame photometric detector (PFD). All the chemicals were purchased from M/s. Merck specialties pvt. Limited and were pesticide residue grade and all pesticide residue standards were purchased from Dr. Erhenstorfer, Germany during 2012. The gas chromatographic analysis was performed under the following conditions (Table 2). A volume of 1 ml sample was injected into the GC; peaks were identified by comparing their retention times (Table 3) with those of standards under the same injection conditions.

The peak areas of the various peaks whose retention times coincide with the standards were extracted on their corresponding calibration curves to obtain the concentrations.

Results and Discussion

A total of 48 fodder samples collected from all the six zones of Musi river belt and were analyzed for OPPs residues. Concentration of various residues in each sample was calculated (in mg/kg sample). In the present study, the average recoveries of OPPs in fodder were 91.27% at 0.05 ppm and 94.67% at 0.1 ppm. The efficiency of extraction methodologies were evaluated based on the recoveries of residues and a recovery of 75-102% is considered as acceptable (Tsiplakou *et al.,* 2010)

Hence, the extraction procedures employed in these experiments were efficient in recovering the maximum amount of residues present in the samples. The elute pattern of various OPPs (0.05 ppm) (Figure 2) along with specific retention time are depicted in Figure 2 for ECD and Figure 3 for pulsated flame photometric detector (PFPD). The limit of detection and limit of quantification for OPPS was 0.05 ppm and 0.05 ppm respectively for both ECD and PFPD.

Fodder samples of Zone II,III,IV & VI of Musi river showed the residues of phorate at concentration of 0.42 ± 0.001 (0.421-0.428), 0.05 ± 0.02 (0.026-0.101), 0.27 ± 0.23 (0.01-0.561) and 0.36 ± 0.01 (0.369-0.385) ppm respectively (Figure 4) (Table 4), Other Organophosphorus compounds collected from zone I, II, III, IV, V and VI were below detection limit in all other fodder samples in the present study.

Feed safety is equally important as food safety as both are directly linked when production of food of animal origin is concerned. Presence of pesticide residues are often reported in different feed resources (Kang *et al.,* 2002; Sharma *et al.,* 2005; Nag, 2006).

Increased use of pesticides in crop protection and control of vector borne diseases during recent years has increased the possibility of feedstuff contamination to high level and the consequent exposure of livestock to these products through feed (Agnihotri, 1999).

Many workers have reported the presence of pesticide residues in the animal feed samples in India (Battu *et al.,* 1996; Singh *et al.,* 1997; Unnikrishnan *et al.,* 1998; Gupta *et al.,* 2000; Sharma *et al.,* 2002). FDA analyzed 545 mixed feed rations for organohalogen and OP pesticides and reported that 88 (16.1%) samples did not contain detectable pesticide residues and in 457 (83.9%) samples with detectable pesticide levels (Lovell *et al.,* 1996).

Though pesticides are not used during cultivation of fodder grasses, detection of residues of pesticide could be due to their absorption from the soil contaminated through previous applications or through water used for irrigation (Unnikrishnan *et al.,* 1998). In a study on detection of residual levels of fenitrotion Lactating dairy cows were fed 50 mg/kg of fenitrothion (dry weight basis) in the feed for 29 days. No residues of fenitrothion appeared in milk by Snelson *et al.,* (1979) and in another study Silage prepared from corn treated at up to 3.36 kg/ha of fenitrothion was fed to lactating dairy cattle for 8 weeks, Fenitrothion was not detected in the milk in Sumithion Technical Manual.

Table.1 Selected zones and covered areas along the Musi river belt, Telangana, India

Zone	Areas covered along Musi river belt
I	Attapur, Langer House, Upper pally, Kishan Bagh,Bahadurpura, Puranapool, Budvel, High court.
II	Chadhar ghat, Malakpet,Morarambagh, Golnaka, Amberpet, Ramanthapur, Nagole, Uppal.
III	Peerzadiguda, K. singaram, Thimaiguda, Pratapa singaram, Korremulla, Bacharam, Bandaraviral, Chinna raviralla.
IV	Pillai Palli, Rudravelly, Brahmanapally, Venkiryala, Edulabad, Nadama Khada, Shivareddy gudem, Alinagar.
V	Indriyala, D.R.palli, Wankamamidi, Shaligowraram, Dharmaram, Chittur, Jajireddygudem, Manimadde.
VI	Musi reservoir, Yendlapally, Kasarabad, Beemavaram, Dasaphad, M.gudem, Irkigudem, Wazirabad.

Table.2 Details of GC operating parameters

GC	GC-Schimadzu 2010
Column	VF-1ms capillary column 30 m length, 0.25 mm internal diameter, 0.25 mm film thickness; 1% methyl siloxane
Column oven (°C)	260 (isothermal)
Detectors	ECD FPD
Detector temperature (°C)	280
Injector temperature (°C)	260
Injector status	Front injector type 1177 split/splitless Split ratio: 1:5
Carrier gas	Nitrogen, Iolar II, Purity 99.99%
Carrier gas flow (ml min^{-1})	1 ml/min
Make-up flow (ml min^{-1})	35 ml/min
Total run time (min)	60 min

ECD=Electron capture detector, FPD=Flame photometric detector, GC=Gas chromatography

Table.3 Details of retentions times of different OPPs by ECD and PFPD

Retention time	ECD	PFPD
Dichlorovas	3.947	3.889
Phorate	13.523	13.427
Methyl parathion	20.249	20.090
Fenitrothion	-	21.726
Quinolphos	26.775	26.587
Profenphos	30.796	30.606
Ethion	34.634	34.436
Phosalone	47.747	47.511
ƛ - Cyhalotrin	48.497	-

ECD=Electron capture detector, PFPD=Pulsated flame photometric detector, OPPs= Organophosphorus pesticides

Table.4 Mean residual levels (ppm) of organophosphorus pesticides in fodder samples along Musi river

Samples	Zones	Dichlorovas	Phorate	Methyl parathion	Fenitrothion	Quinolphos	Profenphos	Ethion	Phosalone	Lambda Cyhalothrin
Fodder	Zone I	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	Zone II	BDL	0.42 ± 0.001	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	Zone III	BDL	0.05 ± 0.02	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	Zone IV	BDL	0.27 ± 0.23	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	Zone V	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	Zone VI	BDL	0.36 ± 0.01	BDL	BDL	BDL	BDL	BDL	BDL	BDL

(Each value is mean of 8 replications), Zone I: Attapur, Langer House, Upper pally, Kishan Bagh, Bahadurpura, Puranapool, Budvel, High court, Zone II: Chadhar ghat, Malakpet, Morarambagh, Golnaka, Amberpet, Ramanthapur, Nagole, Uppal, Zone III: Peerzadiguda, K. singaram, Thimaiguda, Pratapa singaram, Korremulla, Bacharam, Bandaraviral, Chinna raviralla, Zone IV: Pillai Palli, Rudravelly, Brahmanapally, Venkiryala, Edulabad, Nadama Khada, Shivareddy gudem, Alinagar, Zone V: Indriyala, D.R.palli, Wankamamidi, Shaligowaram, Dharmaram, Chittur, Jajireddygudem, Manimadde, Zone VI: Musi reservoir, Yendlapally, Kasarabad, Beemavaram, Dasaphad, M.gudem, Irkigudem, Wazirabad, BDL=Below determination level (<0.05)

Fig.1 Research zones along Musi river belt

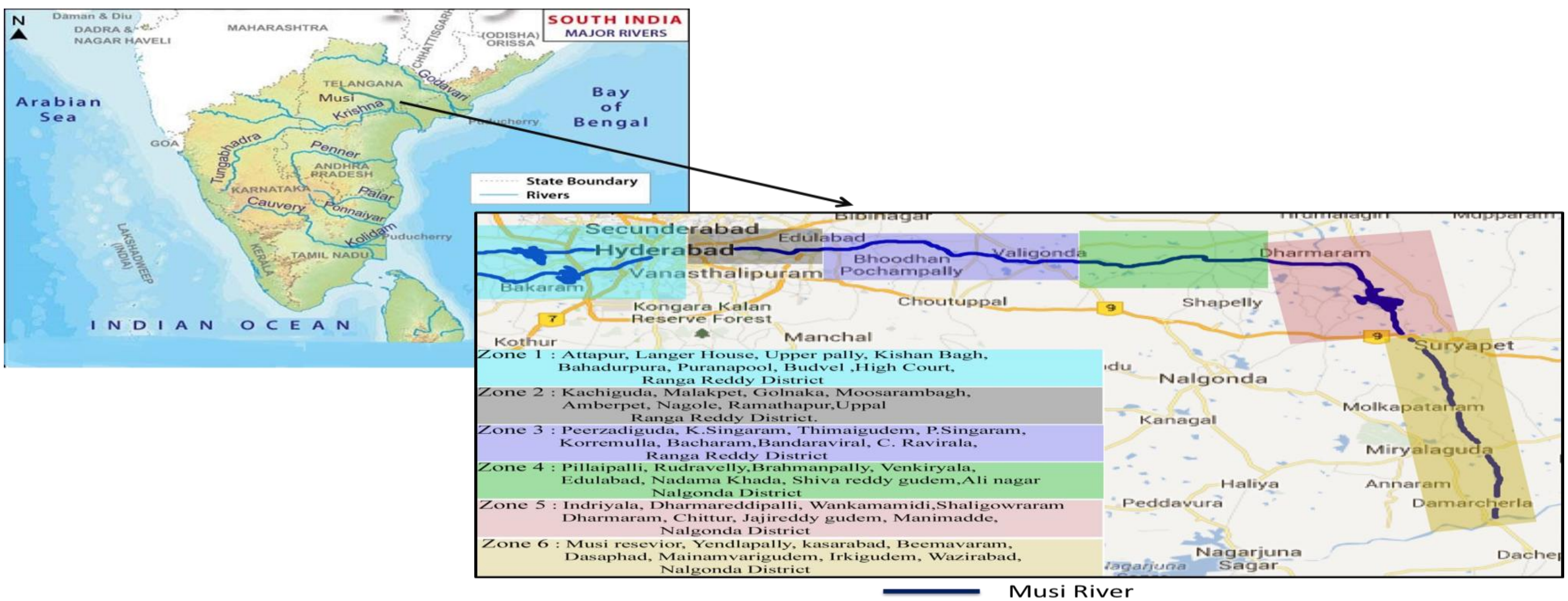


Fig.2 Elution pattern of organophosphorus pesticide standards mixture by electron capture detector

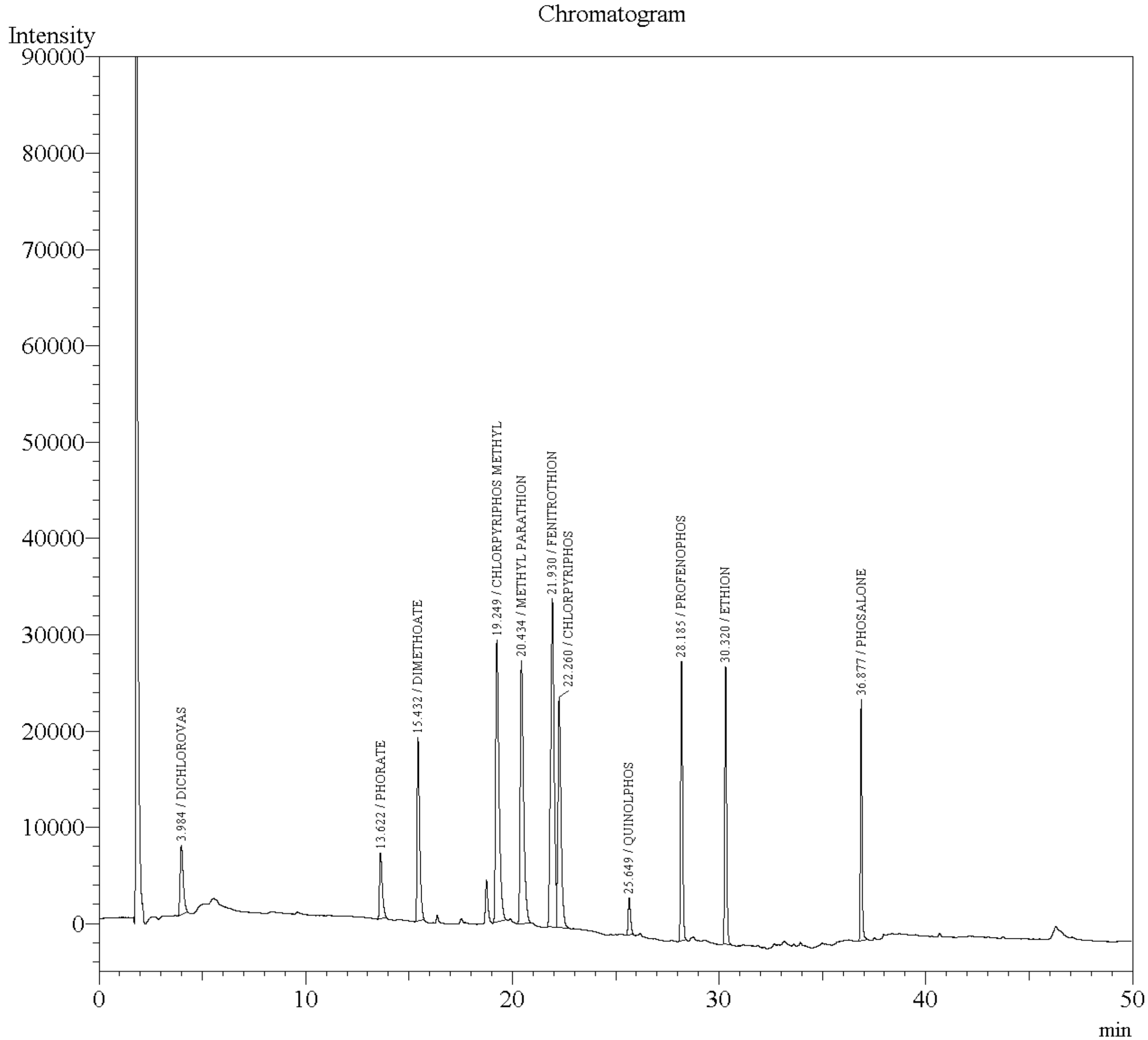


Fig.3 Elution pattern of organophosphorus pesticide standards mixture by pulsated flame photometric detector

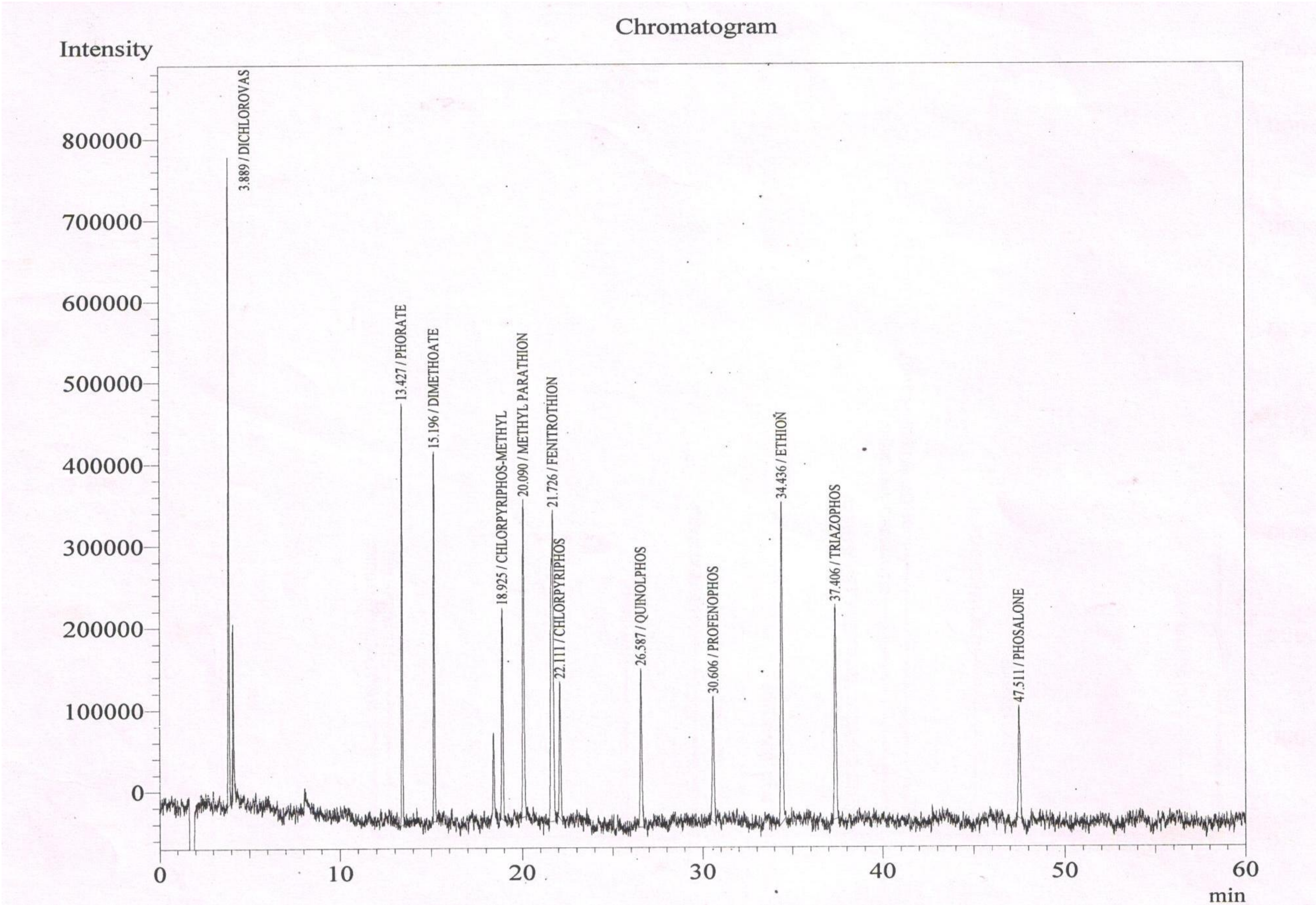
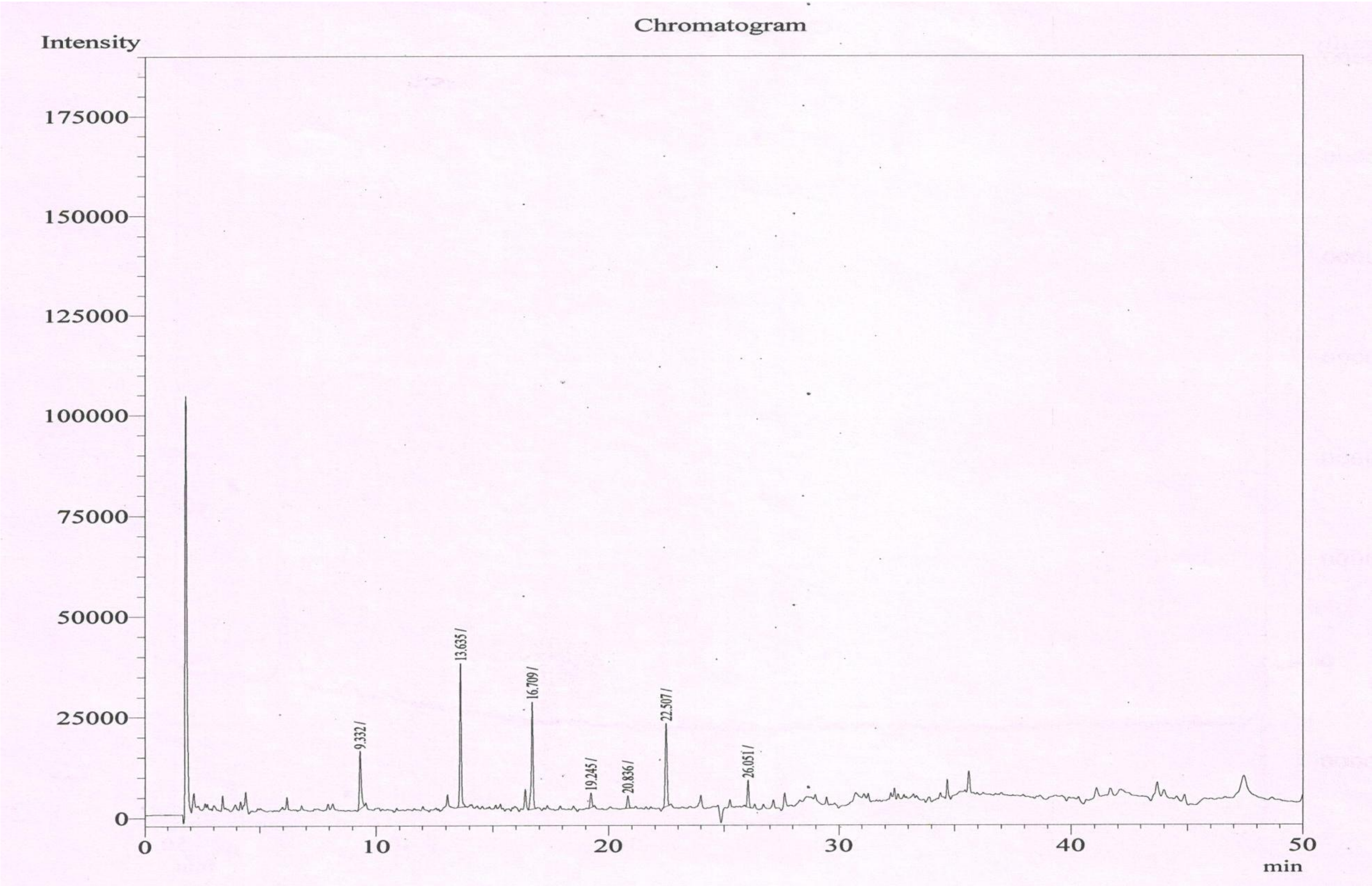


Fig.4 Elution pattern of organophosphorus pesticide residues in fodder samples from zone IV of the Musi river belt



From this study, it can be concluded that all the pesticide residues in fodder samples were below the detection limits except phorate in fodder sample form zone II,III,IV and VI could be detected, it might be due to use of these pesticides on vegetable crops grown on the banks of Musi river belt. The results of OPPs in different samples were detected by ECD and confirmed by PFPD. Further work is needed to determine the bioaccumulation of these toxic elements in the food web and the associated risks to the ecosystem and human health.

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