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Research Article

PESTICIDE RESIDUE DETECTION AND ITS CHROMATOGRAPHIC ANALYSIS IN FEATHERS OF FERAL PIGEON (COLUMBA LIVIA) COLLECTED FROM GRAIN STORAGE GODOWNS OF JALANDHAR, HOSHIARPUR AND LUDHIANA REGIONS OF PUNJAB

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ABSTRACT

The aim of the present study was to investigate whether bird feathers can be used as a non-destructive biomonitoring tool for organic pollutants or not. For this, we analyzed the body feathers of feral pigeons from grain storage godowns of Jalandhar, Ludhiana and Hoshiarpur for persistent Organochlorine residues (Lindane), Organophosphates (Quinalphos), Carbamate (Carbofuran), Synthetic Pyrethroids (Cypermethrin) and Neonicotinoids (Acetamiprid). The result of the study showed that the Acetamiprid, Carbofuran, Lindane, and Quinalphos were undetectable but cypermethrin was detected in feathers of pigeon at all the locations of Punjab and its concentration was 4.7 x 10^{-5} ppm. This might be due the low usage of Acetamiprid, Lindane, Carbofuran and Quinalphos on the crops or could be due to fewer amounts of the samples taken. Although our results suggest that exact concentrations of pesticides in the body cannot be predicted by using feathers, but the bird feathers can give a good estimate of contamination levels of pesticides in a population. So, feathers can be used as a potential non-destructive biomonitoring tool to know the contamination level of pesticides without causing severe damage to the birds.

INTRODUCTION

Birds have extensively been used in the past as biomonitors of environmental contamination with persistent organic pollutants[12]. They are situated high on the food chain, thus accumulating high levels of pollutants, and they are sensitive to environmental changes[8]. The amount of pollutants accumulating within the tissues of birds is related to their diet and corresponding trophic position[4, 15], but also to differences in accumulation among habitats. Because practical and ethical reasons inhibit the sacrifice of free-living animals, methods for non-destructive biomonitoring have been developed[8]. The use of hair, a keratinous tissue, has recently been evaluated as a method for the analysis of pollutants [1, 5, 6, 7, 22]. Since feathers are composed of a keratinous matrix as well, they are potentially useful to study contamination with organic pollutants. In contrast to hair, which is continuously growing, feathers grow only for a certain period of time and are connected to the blood stream (and its circulating pollutants). While many biomonitoring studies on organic pollutants have previously focused on bird eggs, feathers have the advantage that they can be collected irrespective of season, age or sex. Moreover, since one feather can easily be removed from a living bird without causing severe damage, this technique could be valuable as a non-destructive biomonitoring tool for endangered species. Bird feathers have been used previously for monitoring heavy metals in numerous studies, but the use of feathers as monitors of organic pollutants has only recently been investigated[10]. Therefore, a study was performed on feral pigeons to know the level of contamination of organic pollutants. The Acetamiprid, Lindane, Cypermethrin, Carbofuran and Quinalphos were taken for the study and all the pesticides were found undetectable in feathers of pigeon except Cypermethrin.

MATERIALS AND METHODS

Study area

The state of Punjab located in North West India, bordering Pakistan, extends from 29°32' North and 73°55' east. It is surrounded by the Indian states of Jammu and Kashmir in the north; the hilly state of Himachal Pradesh in the east; and by the state of Haryana and Rajasthan in the south. It covers a geographical area of 50,362 square kilometres and is one of the smallest states in India.

The study area comprises of one district of the Malwa region in Punjab namely, Ludhiana and from the Doaba region the districts are Jalandhar and Hoshiarpur which have been chosen on the basis of cropping pattern and pesticide application.

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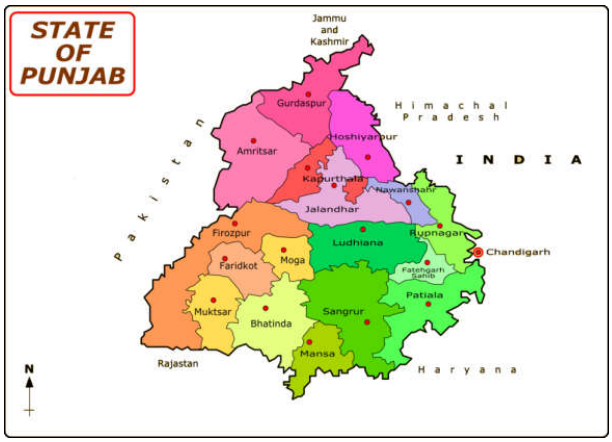


Figure 1 Location of the study areas of Punjab which are Jalandhar, Hoshiarpur and Ludhiana

Statistical analysis

The data obtained from the above experiments was subjected to statistical analysis. These calculations are based on biological statistics and the values are expressed as mean ± standard error of mean (S.E.). The differences in the concentrations of pesticides among the study areas were evaluated by one-way analysis of variance (ANOVA).

Chemical analysis

All the glassware was thoroughly washed with extran (cleaning agent) and after proper rinsing with distilled water, dried in hot air oven. It was again rinsed with solvents to make them free from residual contamination, if any, before use. To remove phthalate esters that interfere in determinations using electron capture detector (ECD), sodium sulphate was heated for 2 hr in a muffle furnace at 400°C. It was stored in a clean stoppered glass bottle and kept in a desiccator for further use. 200 g of florisil (60-100 mesh) was taken in one litre Erlenmeyer flask and 500 ml of dichloromethane was added to it. The contents of the flask were shaken for 30 minutes and filtered through fluted filter paper. The washed florisil was transferred to another one litre Erlenmeyer flask containing 500 ml acetone. After stirring again for 30 minutes, the contents were filtered through fluted filter paper and dried in hot air oven at 130°C for 2h. After that florisil was stored in a clean stoppered glass bottle and kept in a desiccator for further use.

Figure 2 Feathers of pigeon

The stock solutions of Acetamiprid, Carbofuran, Cypermethrin and Quinalphos pesticides were prepared by dissolving standards in acetonitrile and of Lindane was prepared in acetone and stored in a freezer at -20°C. Standard solutions for injection in the gas chromatography (GC) and High Performance Liquid Chromatography (HPLC) were also prepared by dissolving stock solutions in acetonitrile and acetone respectively.

One gram of feather samples were air dried and were crushed to powdered form with the help of pestle and mortar and were dipped with 100 ml acetone for the estimation of Lindane and were dipped in 100 ml of acetonitrile for the estimation of Acetamiprid, Carbofuran, Cypermethrin and Quinalphos and were kept overnight. Filtered the extracts with rinsing of acetone and acetonitrile respectively in 500 ml separating funnel containing 100 ml of distilled water of 5% sodium chloride solution. 50ml of dichloromethane was added and lower layer was collected. Then rinsed with 50ml of hexane and this step was repeated twice and the organic layer was collected by passing through anhydrous sodium sulphate. The different fractions were combined and treated with 500mg of activated charcoal powder for about 2-3 hours at room temperature. The clear extract so obtained was filtered and concentrated in a rotary to 20 ml.

This elute was concentrated in rotary vacuum evaporator and simultaneously dichloromethane was replaced with hexane. The final volume of the elute was made to 5 ml and stored in labelled centrifuge test tube with stopper in deep freezer for analysis in gas chromatography (GC) and High Performance Liquid Chromatography (HPLC).

The sample extracts were analyzed at Central instrumentation laboratory of NIPER, Chandigarh, using Gas Chromatography for the estimation of Lindane and using High Performance Liquid Chromatography (HPLC) for the estimation of Acetamiprid, Carbofuran, Cypermethrin and Quinalphos.

Experimental conditions of gas chromatography were followed for the estimation of lindanes and the operating parameters were as follows:

For Lindane

Column was MDN-5, *Injection parameters*: temperature-300°C, Injection mode- split: 1:10, *Oven parameters*: 150°C to 300°C (10°C/min) hold 15 min, *Detector parameters*: temperature: 320°C, Detector- FID, flow rate was 1.0 ml/min and Helium was used as carrier gas, and a known volume of 1µl was injected in Dichloromethane.

Experimental conditions of high performance liquid chromatography (HPLC) were followed for the estimation of Acetamiprid, Carbofuran, Cypermethrin and Quinalphos and the operating parameters were as follows:

For Acetamiprid

Column was C-18, Column temperature: 25°C, *Eluent preparation*: Acetonitrile: Water in the ratio of 30:70 + 0.1% Phosphoric acid, flow rate was 1.4 ml/min, detector was UV-250 nm, and injection volume was 10µl.

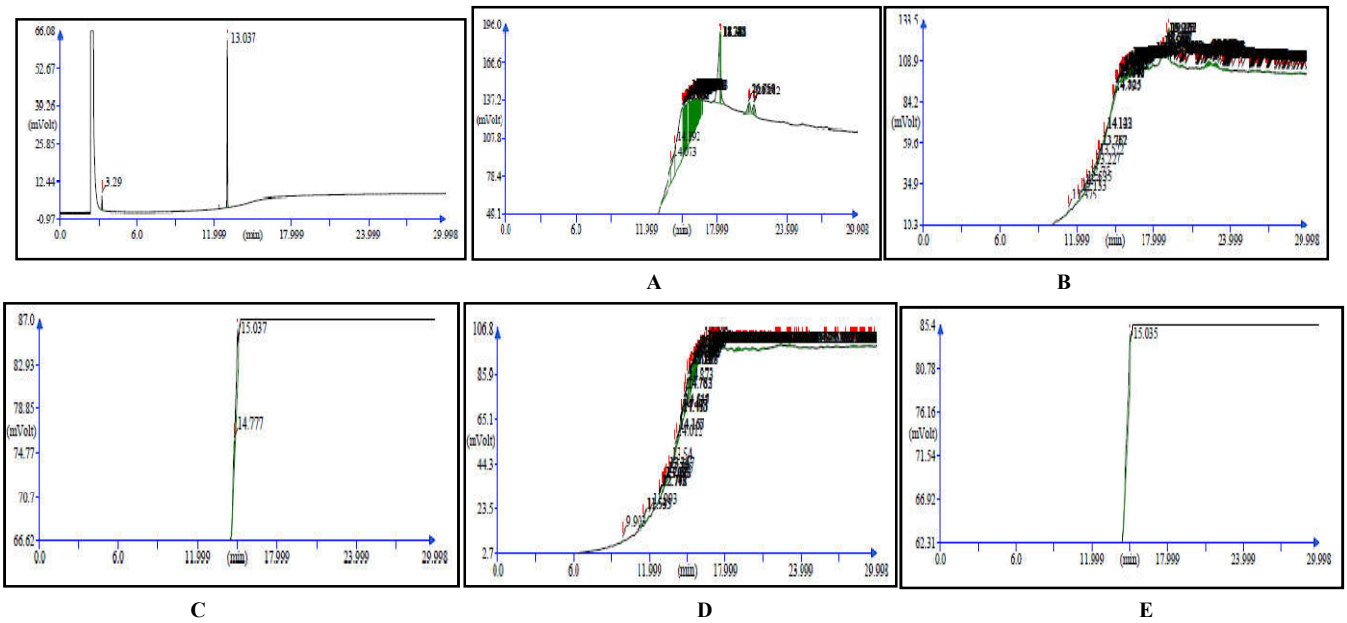
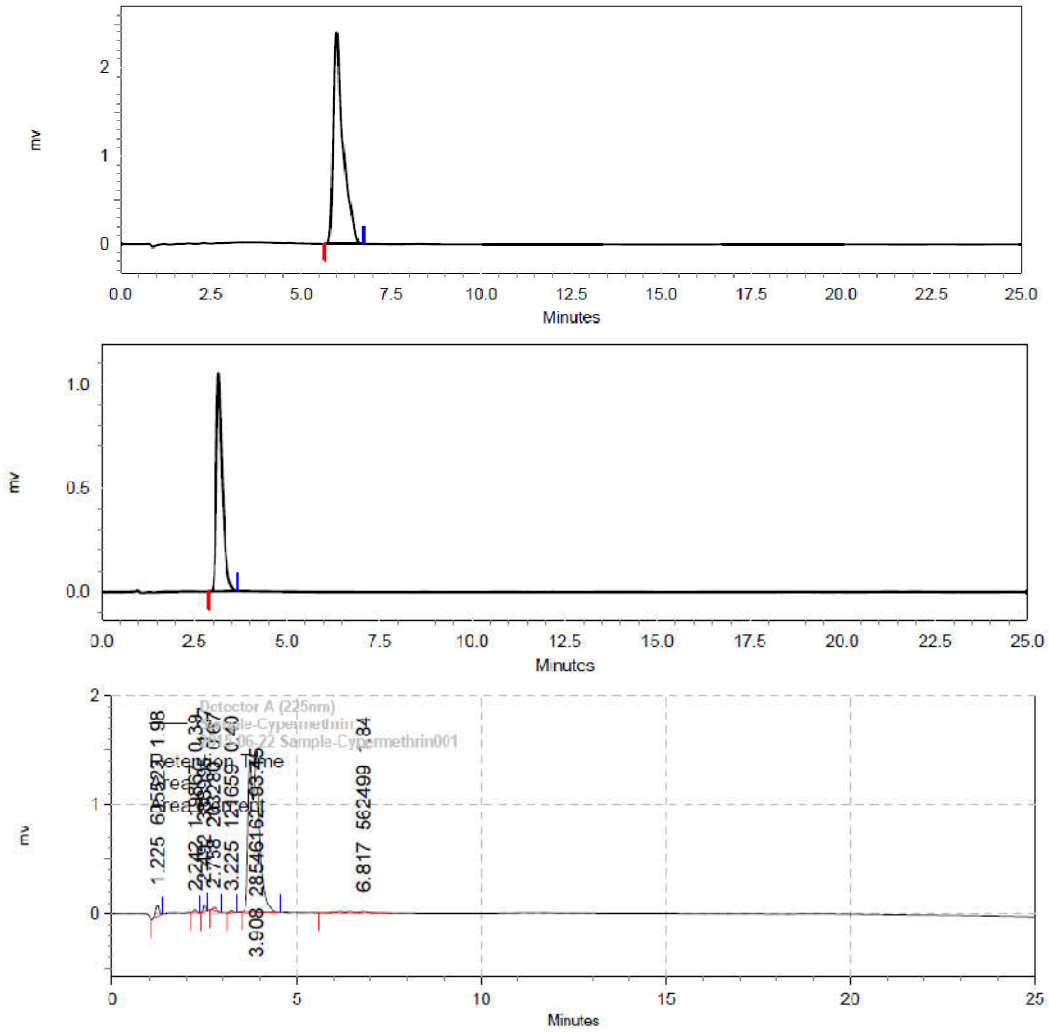


Figure 3 Chromatograms for Lindane (OCs) residue analysis in feathers of pigeon at Ludhiana, Jalandhar and Hoshiarpur

a. Chromatogram of standard solution of Lindane
b. Chromatogram for Lindane residue analysis in pigeons at agronomy farm, PAU, Ludhiana
c. Chromatogram for Lindane residue analysis in pigeons at cold storage, Jalandhar bypass, Ludhiana
d. Chromatogram for Lindane residue analysis in pigeons at cold storage, Mullanpur, Ludhiana
e. Chromatogram for Lindane residue analysis in pigeons at FCI godown, Jalandhar
f. Chromatogram for Lindane residue analysis in pigeons at FCI godown, Hoshiarpur



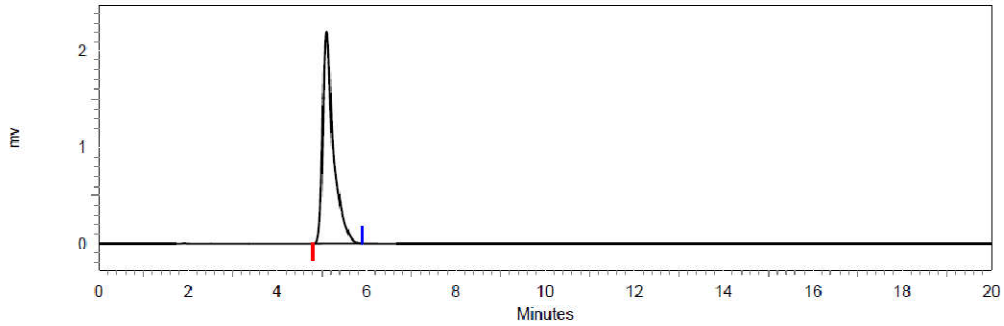


Figure 4 Chromatograms for standard solutions of Quinalphos (OP), Carbofuran (CM), Cypermethrin (SP), Acetamiprid (Neonicotinoid) residue analysis in feathers of pigeon at Ludhiana, Jalandhar and Hoshiarpur

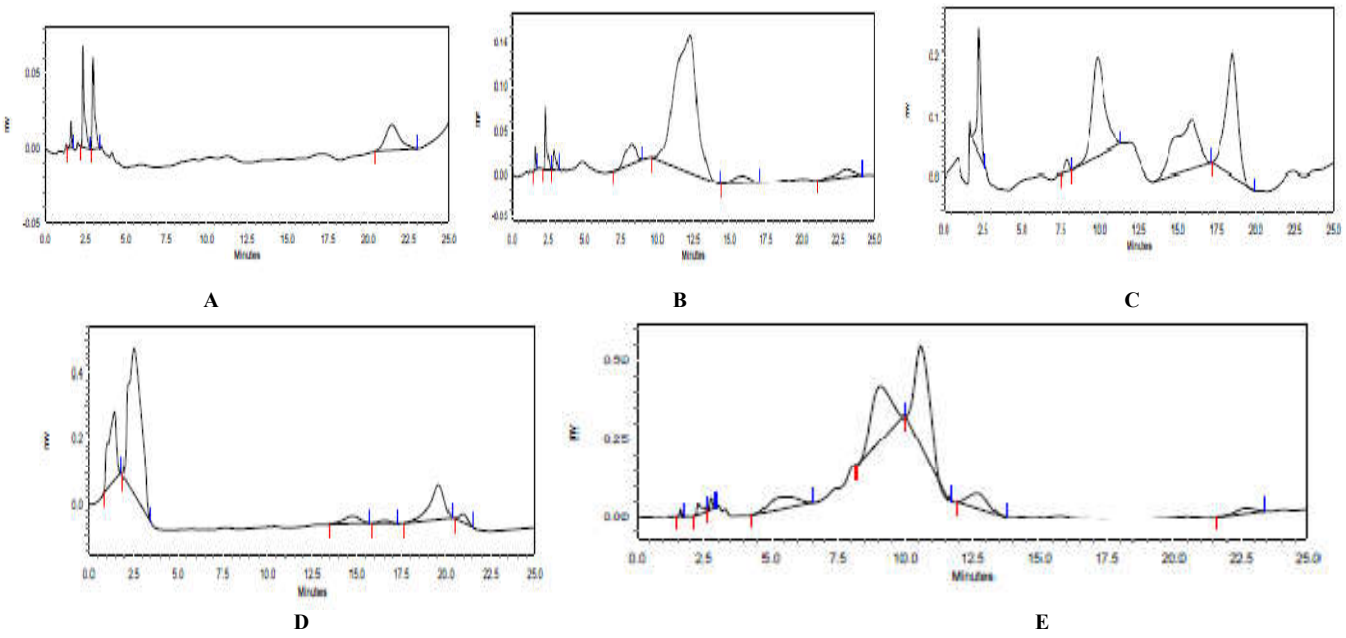


Figure 5 Chromatograms for Quinalphos (OP), Carbofuran (CM), Cypermethrin (SP), Acetamiprid (Neonicotinoid) residue analysis in feathers of pigeon at Ludhiana, Jalandhar and Hoshiarpur

a. Chromatogram for Quinalphos (OP), Carbofuran (CM), Cypermethrin (SP), Acetamiprid (Neonicotinoid) residue analysis in pigeons at agronomy farm, PAU, Ludhiana
b. Chromatogram for Quinalphos (OP), Carbofuran (CM), Cypermethrin (SP), Acetamiprid (Neonicotinoid) residue analysis in pigeons at cold storage, Jalandhar bypass, Ludhiana
c. Chromatogram for Quinalphos (OP), Carbofuran (CM), Cypermethrin (SP), Acetamiprid (Neonicotinoid) residue analysis in pigeons at cold storage, Mullanpur, Ludhiana
d. Chromatogram for Quinalphos (OP), Carbofuran (CM), Cypermethrin (SP), Acetamiprid (Neonicotinoid) residue analysis in pigeons at FCI godown, Jalandhar
e. Chromatogram for Quinalphos (OP), Carbofuran (CM), Cypermethrin (SP), Acetamiprid (Neonicotinoid) residue analysis in pigeons at FCI godown, Hoshiarpur

For Carbofuran

Column was C-18, Column temperature: 25°C, *Eluent preparation*: Acetonitrile: Water in the ratio of 40:60, flow rate was 1.4 ml/min, detector was UV- 272 nm, and injection volume was 20µl.

For Cypermethrin

Column was C-18, Column temperature: 25°C, *Eluent preparation*: Eluent A Acetonitrile, Eluent B Water + 0.1% Phosphoric acid (gradient), flow rate was 1.4 ml/min, detector was UV- 225 nm, and injection volume was 10µl.

For Quinalphos

Column was C-18, Column temperature: 25°C, *Eluent preparations*: ratio of 70:30 of acetonitrile: water, flow rate was 1.4 ml/min, Detector was UV- 235 nm, and Injection volume was 10µl.

Calculations

The formula used for the quantification of residues was:

$$\text{Residues}\left(\frac{mg}{kg}\right) = \frac{\text{Peak area of the sample x ng of insecticide standard injected} \times \text{final volume of the sample extract (ml)}}{\text{peak area of the standard} \times \text{volume of the sample (µ) injected} \times \text{weight of the sample (g)}}$$

In general, the volumes of the sample extract for injection was so chosen that it gave approximately the same area or that of the same peak height obtained with the standard solutions.

RESULTS AND DISCUSSION

Lindane (OCs) residues in feathers of pigeon

The Lindane was taken as a representative of organochlorines and its level was analyzed in feathers of pigeon. There was no Lindane residue in the feathers (Table 1) of pigeon which may be because of their undetectable levels in 1 gram samples of

bird feathers or these may not be present at all in the feathers of these pigeon.

Table 1 Level of Lindane residue (ppm) in feathers of pigeon at different locations of Punjab

Locations	Concentration (ppm) of Lindane residues in feathers
Standard solution of Lindane	0.1 ng
PAU, Ludhiana	0.00
Jalandhar Bypass, Ludhiana	0.00
Mullanpur, Ludhiana	0.00
Jalandhar, FCI Godown	0.00
Hoshiarpur , FCI Godown	0.00
CD At 5%	0.00

Values are expressed as ppm
CD (5%) NS

Quinalphos and Carbofuran residues in feathers of pigeon

The Quinalphos was taken as a representative of organophosphates and Carbofuran as representative of carbamates. Both the pesticides were undetectable in the feathers (Table 2).

Table 2 Level of Quinalphos and Carbofuran residues (ppm) in feathers of pigeon at different locations of Punjab

Locations	Concentration (ppm) of Quinalphos and Carbofuran residues in feathers
Standard solutions of Quinalphos and Carbofuran	0.1 ng
PAU, Ludhiana	0.00
Jalandhar Bypass, Ludhiana	0.00
Mullanpur, Ludhiana	0.00
Jalandhar, FCI Godown	0.00
Hoshiarpur , FCI Godown	0.00
CD At 5%	0.00

Cypermethrin residues in feathers of pigeon

The Cypermethrin was taken as representative of synthetic pyrethroids and was also analysed and found to be absent 1 gram of feathers of pigeon except at the location of Jalandhar FCI. The value of Cypermethrin detected in feathers was 4.7 x 10^{-5} ppm (Table 3).

Table 3 Level of Cypermethrin residue (ppm) in feathers of pigeon at different locations of Punjab

Locations	Concentration (ppm) of Cypermethrin residues in feathers
Standard solution of Cypermethrin	0.1 ng
PAU, Ludhiana	0.00
Jalandhar Bypass, Ludhiana	0.00
Mullanpur, Ludhiana	0.00
Jalandhar, FCI Godown	4.7 x 10^{-5}
Hoshiarpur , FCI Godown	0.00
CD At 5%	0.00

Acetamiprid residues in feathers of pigeon

The Acetamiprid was taken as representative of neonicotinoids and was also analysed and found to be absent in 1 gram of feathers (Table 4) of pigeon at all the location of Punjab.

Table 4 Level of Acetamiprid residue (ppm) in feathers of pigeon at different locations of Punjab

Locations	Concentration (ppm) of Acetamiprid residues in feathers
Standard solution of Acetamiprid	0.1 ng
PAU, Ludhiana	0.00
Jalandhar Bypass, Ludhiana	0.00
Mullanpur, Ludhiana	0.00
Jalandhar, FCI Godown	0.00
Hoshiarpur , FCI Godown	0.00
CD At 5%	0.00

The current study didn't find any gross observation on Acetamiprid, Carbofuran, Lindane and Quinalphos pesticide residues in the feathers of pigeons in Punjab but it revealed the detection of only Cypermethrin in the feathers. It may be because of more use of cypermethrin in the cropland areas of Punjab as most of the pollutants can travel long distances thus act as trans-boundary pollutants which caused widespread population decline of raptorial birds. The birds have also been shown to be more suspectible than mammals to many pesticides because of their relatively inefficient system of detoxification. The non-detectability of Lindane, Quinalphos, Carbofuran and Acetamiprid in feathers of pigeon at all the locations present work may be because of their less use for insect pest control in Punjab. These pesticides are not banned but if their concentration was found to be more than recommended value, then it could cause harm to the birds.

Although enough information is available about cypermethrin toxicity from different regions of the world and for different animals, yet little work has been accomplished on cypermethrin toxicity in wild and domestic pigeon.

Cypermethrin is practically non-toxic to birds, but is highly toxic to fish and aquatic invertebrates. This is mainly because it is metabolized and eliminated significantly more slowly by fish than by mammals or a low avian and mammalian toxicity and important in birds and classified as a Schedule poison in the Standard for the Uniform Scheduling of Drugs. Although the mechanism of toxicity of pyrethroids has not fully explored, various opinions have been put forward. CY can induce oxidative stress in blood cells or may accrue in cell membranes and disturb structure of membrane.

Cypermethrin can impact bird populations by killing insect larvae normally used for food. A study of nesting success of blue tits following an aerial application of cypermethrin in an oak forest found a nearly 100 percent mortality of the caterpillars used as food by the blue tits. When cypermethrin spraying coincided with tit egg hatch and the early nestling stage, the result was an increase in nestling deaths, a decrease in the proportion of successful nests, and a decrease in weight of the surviving nestlings[17].

CONCLUSION

Although our results suggest that exact concentrations in the body cannot be predicted using feathers, but bird feathers can give a good estimate of contamination levels in a population and as such are a potential non-destructive biomonitoring tool for organic pollutants.

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