



# Estimation of Organophosphates in Soil and Water Samples of Noida Region

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## Abstract

Chlorpyrifos, an organophosphate pesticide is extensively used in agriculture and thus its residues are found in soil as well as water and in the surrounding areas. The presence of these insecticides in the environment result as a significant source of ecological contamination, biomagnification and toxicity. Ten samples of soil and water were collected from the different areas like river, pond, agriculture field etc. and this samples were spiked with Chlorpyrifos to estimate the degradation of pesticides with time and pesticide is extracted by two different methods i.e solvent and Microwave assisted extraction and then Thin layer chromatography (TLC) and Gas chromatography (GC) were used to determine the extracted Chlorpyrifos residue. The study clearly shows that chlorpyrifos residues are found in Noida & Greater Noida soil and water samples. Degradation studies showed clearly that even after 12 days Chlorpyrifos is not completely degraded in soil and converted into its hydrolytic product 3,5,6-Trichloro-2-Pyridinol and the final degradation of Chlorpyrifos was 99 -100%. Thus, the presence and degradation of chlorpyrifos residues varies with the source and time period after spray. From this data it is concluded that chlorpyrifos is a pesticide used in the Noida region and the gas chromatography gives more clear results because it detects even small amount of pesticide residue but for Thin layer chromatography higher concentration of pesticide is required.

**IndexTerms - organophosphate, biomagnification, Degradation, chlorpyrifos, contamination.**

## INTRODUCTION

Organophosphate (OP) is a human-made pesticide and poisonous to insects and mammals. They are used in agriculture, and home gardens. Organophosphate insecticides work by damaging an enzyme in a body called acetylcholinesterase. These enzymes control the nerve signal in the body and damage to these enzymes kill pests and cause unwanted side effects to human health [1]. Organophosphorus pesticides (OPPs) enter the soil ecosystem because of direct spraying on the soil surface. The pesticides are mixed with the soil. Rain carried soil with them and mixed with the water. Due to this water bodies are also contaminated with pesticides which also disturbed the aquatic life [2]. Chlorpyrifos has been extensively used in the agricultural sector for controlling insects, infestations of crops, and rapid growth of plants, cotton, fruits and vegetables [3]. The hydrolysis product of chlorpyrifos 3,5,6-chloro-2-pyridinol (TCP) is a major metabolite of chlorpyrifos.

The aim of this project is to contributing to the knowledge of the organophosphate like chlorpyrifos and its environmental impact on the soil and water in the Noida region. The purpose of this project is to find the concentration of pesticide residue which remains in the soil, water bodies, vegetable and fruits or to avoid possible risk to human health, by preventing people from drinking that water and using it for irrigation. These pesticide residues act like slow poison, slowly killing human individuals. Contamination of aquatic and terrestrial ecosystems with toxic pesticides causes Bioaccumulation and Biomagnification of the toxic material and is an environmental problem of public health concern [4]. The techniques used in this study to detect and estimate chlorpyrifos residue are chromatographic based - methods such as Gas chromatography (GC) and Thin layer Chromatography (TLC) [5,6].

## MATERIAL AND RESEARCH METHODOLOGY

### 2.1 Study area

Noida is a planned city in India and it's a Northern state of Uttarpradesh, its Geographical areas is around 1442 km<sup>2</sup>

## 2.2 Chemicals and reagents.

Pesticides selected for the study of market samples is chlorpyrifos ( $C_9H_{11}Cl_3NO_3PS$ ), its IUPAC name is O,O-diethyl O-3,5,6-tri chloropyridin-2-yl phosphorothioate, boiling point is  $160^\circ C$  and melting point  $43^\circ C$ .

## 2.3 Preparation of standard and working solutions for soil and water.

0.2% standard was prepared by adding  $0.1\mu L$  of chlorpyrifos in 50 ml of Distilled water and two solvent extraction solutions were used.

Solution 1: Solution 1 is used by adding Sodium Chloride (1g), sodium Sulphate (1g), Ethyl acetate (10ml) in 5g/5ml of soil and water.

Solution 2: Solution 2 is used by adding Acetone : Hexane in (1:1)(10ml), sodium Chloride (1g), Sodium Sulphate (1g) in 5g/5ml of soil and water.

The extraction of the pesticide residues was done using the above given solutions and reagents.

## 2.4 Sampling

Total 10 samples of Soil and water were collected from agricultural plots and ponds in clean bottles and boxes. Samples were kept and wrapped in a clean paper bag. The samples were then stored in a cold and dry area for further analysis.

## 2.5 Application of pesticides on the soil & water samples.

To compare the natural samples obtained from the environment, a small part of the samples was spiked or sprayed with the pesticide also. The samples were sprayed with the pesticides chlorpyrifos. The spray consists of 0.2% of the pesticide's solution. Soil and water sample has been taken which was spiked with 0.1 microliter chlorpyrifos. The samples were taken for 2, 5, 7, 9, and 12th days and kept in a separate beaker by covering it with aluminum foil. The same procedure was followed to take 5 samples of soil and water from different areas to show the presence of chlorpyrifos.

## 2.6 Extraction:

### Extraction of Chlorpyrifos from soil and water by the Ethyl Acetate / Acetone : Hexane methods:

5 g / 5ml of soil / water + 1gm of sodium chloride + 1gm of sodium sulphate + 10 ml of Ethyl acetate or Acetone and Hexane were mixed with the help of a mortar pistol. The Mixture was heated in a microwave for 4-5 minutes for proper extraction. Centrifugation was done at 10000 rpm for 110 minutes. Supernatant was collected and evaporated in a tube. The residue remaining at the bottom was dissolved in 500 ul of Acetone and Hexane and stored at  $4^\circ C$ . Repeat this Extraction process for all the soil and water samples.

## 2.7 Detection and Estimation of Chlorpyrifos residue

Detection of Chlorpyrifos by TLC plate:

The extracted samples were examined on the TLC plate for detection of Chlorpyrifos residues in the samples. The TLC plates were made using silica gel (Merck) slurry. They were first air dried and then oven baked for 2 hours before use for activation. The solvent used for separation of Chlorpyrifos on the TLC plates was Butanol: acetic acid: water (5:1:1). The chlorpyrifos spots or bands were obtained on the TLC spots, which were visualized after drying of TLC plates.

The positions of noticeable components or spots were marked. The Rf value was then calculated to detect the presence of Chlorpyrifos residue in the samples.

$$R_f = \frac{\text{the distance traveled by the solute}}{\text{The distance traveled by the solvent.}}$$



Fig: 1 and 2 shows the presence of chlorpyrifos in soil sample in 12<sup>th</sup> day.



Fig: 3 and 4 shows the presence of chlorpyrifos in water sample in 5<sup>th</sup> day.

**Estimation of Chlorpyrifos residue by Gas Chromatography :**

Gas chromatography is widely used for the qualitative and quantitative analysis of a large number of low polarity compounds because it has high sensitivity. The GC instrument used in this study was a gas (flow rate = 4 mL/min) was used as carrier gas for the separation. All standards and samples were directly injected (1-2 µl) to the GC system with column temperature pre-incubated at 160 °C. The oven, injector and detector temperatures were fixed at 200<sup>0</sup>C degrees. To evaluate the chlorpyrifos residue using this device, pure Chlorpyrifos standards were used.

There are some figures which shows the estimation of Chlorpyrifos residue by Gas Chromatography:-

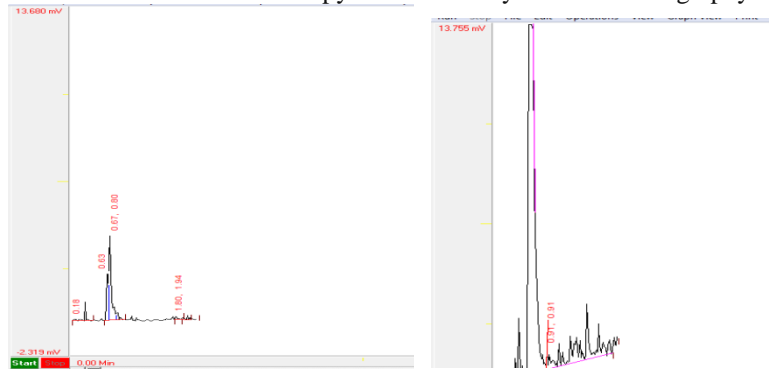


Fig: 5 Shows the presence of Chlorpyrifos in soil in 12 th day by Extraction process 1 and 2.



Fig: 6 Shows the presence of Chlorpyrifos in water in 12 th day by Extraction process 1 and 2.

**RESULTS AND DISCUSSION**

The present study was undertaken to detect and estimate the Chlorpyrifos residue present in the soil and water bodies near the agricultural fields of Noida. To further analyse and understand the degradation pattern of Chlorpyrifos in soil and water naturally, samples were spiked with the pesticide and incubated for a different number of days. After these intervals the chlorpyrifos residue was estimated and detected. The detection and estimation were done by TLC and Gas Chromatography.

**Analysis through TLC :** After the extraction, the TLC plate was loaded with Chlorpyrifos. There were 30 samples in one solvent system, i.e Butanol: acetic acid : water in 5:1:4. with different volumetric ratios. The R<sub>f</sub> value of the Chlorpyrifos extracted from 30 samples was nearly the same as the standard used. The Chlorpyrifos residue was detected in both soil and water samples which were extracted by different extraction methods using one solvent system for TLC.

Table 1: Chlorpyrifos residue detected in Soil & Water samples (Spiked with 0.2% chlorpyrifos)

S no.		Days	(EXTRACTION METHOD) Ethyl Acetate (Rf1)	(EXTRACTION METHOD) Acetone : Hexane (Rf2)
1.	Soil Sample	2-days	Residue Present	Residue Present
	Water Sample		Residue Present	Residue Present
2.	Soil Sample	5-days	Residue Present	Residue Present
	Water Sample		Residue absent	Residue absent
3.	Soil Sample	7-days	Residue Present	Residue Present

	Water Sample		Residue absent	Residue absent
4.	Soil Sample	9-days	Residue Present	Residue Present
	Water Sample		Residue absent	Residue absent
5.	Soil Sample	12-days	Residue Present	Residue Present
	Water Sample		Residue absent	Residue absent

Abbreviations: Rf1 = Sodium chloride, sodium sulphate, Ethyl acetate and Rf2= Acetone : Hexane, both are the extraction methods.

The results show that the Hexane : Acetone method with MAE is a better extraction method for soil as even at 12 day chlorpyrifos residue is detected and chlorpyrifos degrades faster in water than the soil.

Table 2 : Chlorpyrifos residue detected in Soil samples and water samples ( direct) Collected from fields of Noida

S no.	Samples	(EXTRACTION METHOD) Ethyl Acetate (Rf1)	(EXTRACTION METHOD) Acetone : Hexane (Rf2)
1.	Soil Sample 1	Residue Absent	Residue Absent
	Water Sample 1	Residue present	Residue Absent
2.	Soil Sample 2	Residue Absent	Residue Present
	Water Sample 2	Residue present	Residue absent
3.	Soil Sample 3	Residue absent	Residue absent
	Water Sample 3	Residue absent	Residue absent
4.	Soil Sample 4	Residue absent	Residue absent
	Water Sample 4	Residue absent	Residue absent
5.	Soil Sample 5	Residue absent	Residue absent
	Water Sample 5	Residue absent	Residue absent

Abbreviations: Rf1 = Sodium chloride, sodium sulphate, Ethyl acetate and Rf2= Acetone : Hexane, both are the extraction methods.

The results for extraction with both the methods showed that Soil sample 2, contains residue of chlorpyrifos when it's extracted with MAE and water sample 1, 2 contain residue of Chlorpyrifos when it's extracted with Ethyl acetate and was absent in Acetone : Hexane extraction method. From this table it is concluded that chlorpyrifos is used in the Noida region and for detection by thin layer chromatography more concentrated pesticides are required.

#### Estimation of degradation of chlorpyrifos in soil and water samples By Gas chromatography

Soil and water samples that were spiked with chlorpyrifos were incubated for the different intervals of time at room temperature to analyze the process of pesticide degradation in the environment. Gas chromatography was used for estimating the chlorpyrifos residues left after 2Day, 4Day, 7Day, 9 Day, 12 Day intervals when the soil and water samples were left untouched. In Each spiked soil and water sample, the extraction has been performed by two solvents: Ethyl acetate and Hexane: acetone. Gas chromatography uses very little concentration of a compound to be detected thus, it is more sensitive and accurate estimation equipment than TLC.

Table 3: Degradation of Chlorpyrifos in Soil and water samples spiked with 0.2% chlorpyrifos

S no.		Days	(EXTRACTION METHOD) Ethyl Acetate (RT1)  % degradation	(EXTRACTION METHOD) Acetone : Hexane (RT2)  % degradation
1.	Soil Sample	2-days	49.25 %	75%
	Water Sample		89%	88%
2.	Soil Sample	5-days	73.66%	85%
	Water Sample		90.3%	90%
3.	Soil Sample	7-days	78.81%	88.33%
	Water Sample		92.5%	94%
4.	Soil Sample	9-days	91.5%	88%
	Water Sample		96.9%	96%
5.	Soil Sample	12-days	98.85%	96.7%
	Water Sample		99.92%	96%

Abbreviations: Rf1 = Sodium chloride, sodium sulphate, Ethyl acetate and Rf2= Acetone : Hexane, both are the extraction methods.

The degradation percentage of soil sample is 98.85% when it extracted with RT1 and 96.7% in RT2 Extraction method, but in water sample it almost 99.92% in RT1 Extraction method and 96% in RT2 Extraction methods.

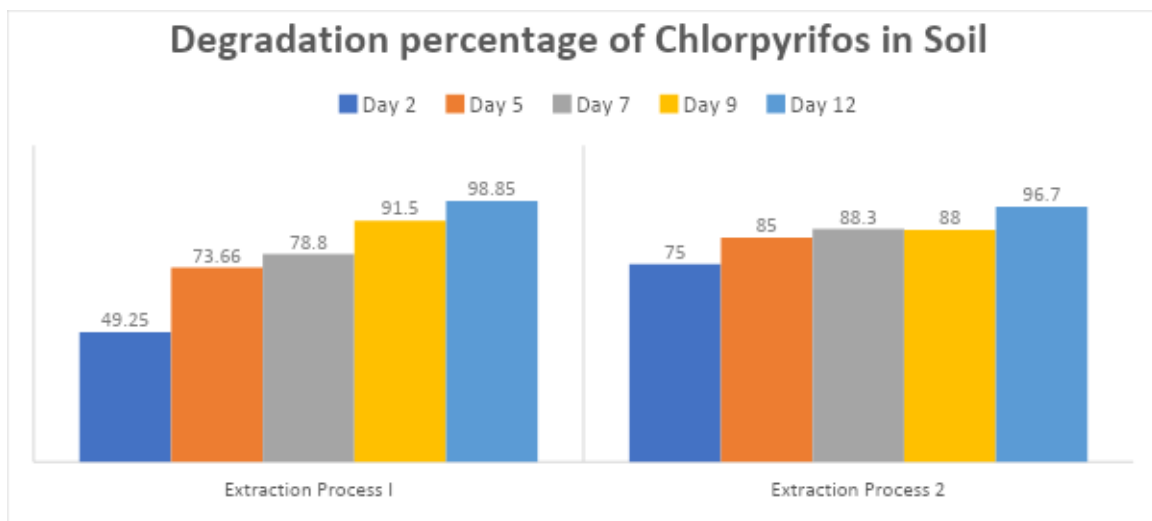


Fig: 7 Degradation Percentage of Chlorpyrifos in Soil over different time period

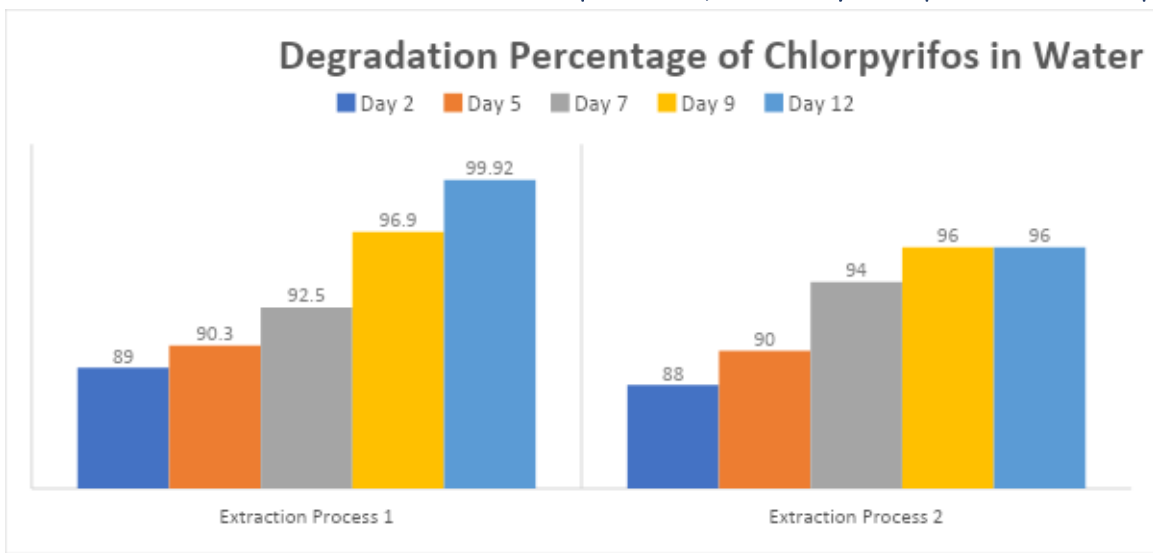


Fig: 8 Degradation Percentage of Chlorpyrifos in Water over different time period

Fig: 7 and Fig: 8 shows that 98 % degradation when extracted with Ethyl acetate for soil sample and shows a final degradation of 97 % was observed by the Hexane: Acetone method. In water sample almost 100% degradation was observed when extracted with Ethyl acetate in 12th day sample. While a final degradation of 96 % was observed by the Hexane: Acetone method. From this degradation percentage theory it's more clear that Chlorpyrifos doesn't degrades even after 12<sup>th</sup> day by both extraction method but in water samples it's almost dregades. So, its clear that chlorpyrifos degrades faster in water than soil.

Table 4: Chlorpyrifos residue detected By GC in Soil samples and water samples Collected from fields of Noida

S no.	Samples	(EXTRACTION METHOD) Ethyl Acetate (RT1)  % Chlorpyrifos present	(EXTRACTION METHOD) Acetone : Hexane (RT2)  % Chlorpyrifos present
1.	Soil Sample 1	23%	11%
	Water Sample 1	---	---
2.	Soil Sample 2	---	---
	Water Sample 2	0.68%	0.54%
3.	Soil Sample 3	0.26%	0.63%
	Water Sample 3	0.25%	2.92%
4.	Soil Sample 4	---	---
	Water Sample 4	0.18%	1.5%
5.	Soil Sample 5	---	---
	Water Sample 5	---	---

Abbreviations: Rf1 = Sodium chloride, sodium sulphate, Ethyl acetate and Rf2= Acetone : Hexane, both are the extraction methods.



The results for extraction with both the methods showed that Soil sample 1 and Soil sample 3 contain residue of chlorpyrifos. The extracted values were different for both the methods but Higher concentration of Chlorpyrifos was extracted from Sample 1 i.e., 25% in case with Ethyl Acetate method. In water samples the water samples 2, 3 and 4 contain residue of chlorpyrifos. The extracted values were less in the range of 0.28 % - 2.92%.

Present study is undertaken as a part of Environmental forensic study, the soil and water samples were collected from different places of Noida and Greater Noida region to estimate and detect the Chlorpyrifos residues. The agriculture areas around the city have a lot of vegetable growing regions. The collected samples of soil showed higher concentration of Chlorpyrifos residue as compared to the water samples. Bit 2-3 samples out of 5 contained the residues of Chlorpyrifos. This clearly showed the toxicity of the soil and water in the region of Noida & Greater Noida.

In the second part of this study soil and water samples were spiked with chlorpyrifos and then were incubated at room temperature to ascertain the degradation percentages. Two different solvents were used for extraction of chlorpyrifos and microwave extraction method was utilized. The extraction showed higher extraction by Hexane: Acetone method and thus gave a lesser degradation percentage as compared to the Ethyl Acetate extraction method.

The Final aspect which is of importance is that the chlorpyrifos pesticide degrades 97 % - 99 % by the end of 12 days in both soil as well as water samples. But degradation of Chlorpyrifos leads to formation of TCP – 3,5,6-Trichloro-2-pyridinol, which is a more persistent and toxic organochemical and stays in the soil and water for a much much longer time.

Widespread pollution in the natural environment is a result of chlorpyrifos' long-term use and broad-spectrum application, causing substantial harm to non-target creatures. Furthermore, because to its high mammalian toxicity, substantial data suggests that chlorpyrifos may impact the endocrine system, respiratory system, cardiovascular system, neurological system, immune system, and reproductive system. In the natural environment, chlorpyrifos can also be transformed to 3,5,6-trichloro-2-pyridinol (TCP), a long-lasting metabolite resistant to microbial destruction. The US Environmental Protection Agency (EPA) classifies TCP as persistent and mobile, with a half life in soil ranging from 65 to 360 days, depending on soil type, climate, and other factors. TCP, as a main chlorpyrifos breakdown product, is more water soluble than its parent molecule, causing widespread pollution of soils and aquatic ecosystems. As a result, it is critical to remove harmful toxins from the environment.

## CONCLUSION

Persistent pesticide usage by Farmers is a major issue resulting in soil and water toxicity as well as Biomagnification in animals and plants. The study thus clearly shows that chlorpyrifos residues are found in Noida & Greater Noida soil and water samples. Degradation studies showed clearly that even after 12 days Chlorpyrifos is not completely degraded and is converted into its hydrolytic product TCP.

The optimized and validated solvent extraction methods along with Microwave assisted extraction procedure was efficient in the determination of chlorpyrifos in soil samples and water samples. The final degradation of Chlorpyrifos was 99 -100% on the 12th day in soil water samples.

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