ORIGINAL ARTICLE

An Investigation of the Dissipation Kinetics of Picloram Herbicide Residues in Lake Water Under Climatic Conditions using a Validated High-Performance Liquid Chromatography with a Photodiode Array Detector Method

Yadagiri Rao Yasala[1], H. Mallesha[1], Tentu Nageswara Rao[2], Y. Prashanthi[1]

[1]Department of Chemistry, Mahatma Gandhi University, Nalgonda, Telangana, India, [2]Department of Analytical Chemistry, Isosphere Biosciences Pvt Ltd., Secunderabad, Telangana, India

Abstract

Aim: Understanding the dissipation kinetics of the picloram herbicide is crucial for assessing its environmental impacts and persistence in aquatic ecosystems. **Materials and Methods:** An experiment was conducted to observe the dissipation behavior of picloram in lake water by introducing uniform concentrations: T0 – Untreated Control, T1 – picloram 24% soluble liquid (SL) at 1 µg/mL, and T2 – picloram 24% SL at 2 µg/mL. The spiked samples were exposed to sunlight. Water samples were collected at various intervals (0.5, 1, 3, 5, 10, 15, and 30 days). All samples were analyzed until the residues fell below the detectable level. The quantification of picloram residues was performed using a validated high-performance liquid chromatography with a photodiode array detector (HPLC-PDA) at a wavelength of 235 nm. **Results and Discussion:** The method's limit of detection was 0.015 µg/mL, and the limit of quantification (LOQ) was 0.05 µg/mL based on recovery. The DT50 (Half-Life) of picloram was calculated through regression analysis of the dissipation data, yielding a DT50 value of 6.63 days for the T1 dose and 6.60 days for the T2 dose. **Conclusion:** This study outlines a rapid HPLC-PDA method for quantifying picloram residues in buffers, utilizing a mobile phase of acetonitrile and 0.1% ortho-phosphoric acid for separation in about 10 min. Validation followed South African National Civic Organization and Environmental Protection Agency guidelines, ensuring satisfactory parameters such as linearity, recovery, precision, and LOQ. The method is applicable for routine monitoring in crops, water, and soil.

Key words: Dissipation, DT50, high-performance liquid chromatography with a photodiode array detector, lake water, picloram, residues

INTRODUCTION

The kinetics of pesticide dissipation in water pertain to the rate and processes by which they decompose or are eliminated from aquatic systems.[1] Key mechanisms of dissipation encompass hydrolysis, photolysis, microbial degradation, sorption, and volatilization. Environmental factors include temperature, pH, oxygen concentration, sediment interactions, and the presence of biofilms and macrophytes.[2] The chemical characteristics of pesticides involve solubility, formulation, and stability. Pesticides that are hydrophilic tend to undergo microbial degradation more readily, while the type of formulation – granular or liquid – can influence dispersion and degradation rates. Modeling the kinetics of dissipation requires the application of first-order kinetics, half-life ($t_1/_2$), and sophisticated models that consider environmental factors and compound-specific isotope analysis. Grasping these processes is essential for

Address for correspondence:
Y. Prashanthi, Department of Chemistry, Mahatma Gandhi University, Nalgonda, Telangana – 508254, India.
Phone: 9010203857.
E-mail: dryprasanthi@gmail.com

Received: 21-08-2025
Revised: 23-09-2025
Accepted: 29-09-2025

developing effective pesticide management strategies and assessing environmental risks.[3-5]

Common pesticides demonstrate considerable dissipation in both water and soil environments as a result of their chemical composition and interactions with the environment. Examples of prevalent pesticides include bentazone, carbofuran, oxamyl, ametryn, deltamethrin, chlorpyrifos, and cartap hydrochloride. Factors that contribute to accelerated dissipation encompass soil type, temperature and moisture levels, sunlight exposure, and the formulation of the pesticide.[6,7] Sandy soils exhibit a more rapid dissipation rate compared to clay soils due to their lower adsorption capacity. Warmer and more humid conditions promote increased microbial activity and hydrolysis processes.[8] Sunlight facilitates photolysis, particularly for pesticides that are applied to the surface.[9,10] Liquid formulations tend to dissipate more quickly than their granular counterparts. Understanding these dissipation patterns is essential for assessing residue levels, ensuring environmental safety, and determining appropriate reapplication intervals.[11-13]

Picloram is a systemic herbicide utilized for the management of broadleaf weeds and woody plants.[14] It is chemically identified as 4-amino-3,5,6-trichloropyridine-2-carboxylic acid and is frequently marketed under brand names such as Tordon and Grazon. Picloram functions as an auxin mimic, interfering with plant development by inducing uncontrolled cell division and elongation.[15] It exhibits high persistence in soil and has the potential to leach into groundwater. Its environmental characteristics include tendencies for leaching, degradation, and selective targeting of broadleaf species.[16] It poses mild toxicity to skin and eyes and can be administered through spraying, injection, or cutting surfaces.[17] The use of picloram has sparked controversy, particularly due to its application in military defoliants like Agent White during the Vietnam War. When contemplating its use or assessing its environmental impact in Telangana, it is advisable to establish a monitoring plan or consider safer alternatives.[18]

Picloram is a highly soluble herbicide that has a considerable environmental impact owing to its significant mobility and persistence. It poses risks of soil and groundwater contamination, threatens surface water, and can adversely affect non-target plants. As a systemic herbicide, it imitates plant hormones, rendering even minimal quantities detrimental. Picloram exhibits moderate toxicity to freshwater fish and slight toxicity to aquatic invertebrates.[19] However, it is virtually non-toxic to birds, mammals, and bees, which makes it less of a concern for terrestrial wildlife.[20] Due to its environmental risks, it is categorized as a restricted-use pesticide in numerous areas.[21] Its application in Telangana, particularly during the monsoon season, carries a high level of risk. Long-term studies on picloram's environmental and ecological effects highlight its persistence in soil, impact on plant communities, and residue behavior.[22] Examples include a Montana study demonstrating strong herbicidal efficacy against spotted knapweed and a Canadian grain production study showing slow dissipation of residue levels.[23]

The current study sought to explore the behavior of picloram residues in lake water when exposed to sunlight.

MATERIALS AND METHODS

Materials

The reference analytical standard for picloram (purity 97.92% [g/g]) was sourced from Ehrenstorfer. The test item, picloram 24% soluble liquid (SL), was acquired from the local market in Hyderabad. Acetonitrile, high-performance liquid chromatography (HPLC) grade water, and ortho-phosphoric acid of analytical reagent grade were procured from Merck India Limited. Lake water samples were collected from Shamirpet Lake in Telangana.

Standard stock solution

Precisely 10.22 mg of picloram reference standard, with a purity of 97.92%, was measured into a 10 mL volumetric flask. The substance was dissolved in 5 mL of acetonitrile, sonicated, and then brought up to the mark with the same solvent. This resulted in a concentration of 1000.74 µg/mL solution, which was subsequently stored in a freezer at −18°C. The stock standard solutions remained viable for use for up to 3 months. Appropriate concentrations of working standards were created from the stock solutions through dilution with acetonitrile, just before the sample preparation.

Sample stock solution

Precisely 85.03 mg of the test substance (purity 24.12%) of picloram was placed into a 20 mL volumetric flask. The substance was dissolved in 10 mL of acetonitrile, subjected to sonication, and then brought up to the mark with additional acetonitrile. This resulted in a concentration of 1025.46 µg/mL solution. The stock sample solution was utilized for the preparation of dose samples (T1 and T2) in lake water.

Application data

Three replication doses were prepared in lake water utilizing a picloram sample stock solution. The first dose was the untreated control, referred to as T0. The second dose, T1, contains a concentration of 1 µg/mL, achieved by fortifying 1.0 mL of the test item stock solution in 1,000 mL of lake water. The third dose, T2, has a concentration of 2 µg/mL, accomplished by fortifying 2.0 mL of the test item stock solution in 1,000 mL of lake water.

Sampling data

The samples were exposed to direct sunlight, and they were prepared by thoroughly mixing and then subsampling 20 mL with a pipette. Water samples were collected on specific days: 0, 1, 3, 5, 7, 10, 15, 20, and 30. The laboratory environment was kept at a temperature ranging from 20°C to 25°C.

Instrument conditions

The HPLC with a photodiode array detector (HPLC-PDA) Waters system, featuring the e2695 separation module and the 2998 PDA detector, was integrated with Empower software. It utilized a reversed-phase C18 analytical column measuring 250 mm × 4.6 mm with a particle size of 5 µm (Phenomenex-C18). The column oven temperature was set at 30°C. A sample volume of 10 µL was injected. Mobile Phases A and B consisted of Acetonitrile and 0.1% ortho-phosphoric acid in a 50:50 (v/v) ratio. The flow rate was maintained at 1.0 mL/min, with the detector operating at a wavelength of 235 nm. The total run time was 10 min, and the retention time for picloram was approximately 4.0 min. This analysis employed the external standard method of calibration.

Method validation

Method validation is essential for ensuring the credibility of analyses. This study focused on the parameters of linearity, recovery, repeatability, limits of detection (LOD), and limit of quantification (LOQ). The method's accuracy was assessed through recovery tests, utilizing samples spiked at concentration levels of 0.05, 0.5, and 3.0 µg/mL. Linearity was evaluated using various known concentrations (0.015, 0.5, 1.0, 10.0, 50.0, and 100.0 µg/mL), which were prepared by diluting the stock solution. The LOD (LOD, µg/mL) was established as the lowest concentration that produced a response of 3.3 times the baseline noise, as defined by the analysis of a control sample. The LOQ (LOQ, µg/mL) was determined based on recovery.

Dissipation kinetics studies

To investigate the dissipation kinetics of picloram residues in water, we utilized concentrations of 1.0 µg/mL (T1) and 2.0 µg/mL (T2) with triplicate replications, alongside control samples for comparative analysis. The samples were subjected to direct sunlight exposure. Aliquots were collected at specified intervals. Throughout the study, water samples were gathered, with temperatures ranging from 25 to 43°C. A 0.45 mm polytetrafluoroethylene (PTFE) membrane filter was used to filter the samples obtained at various sampling times. The resulting filtrates were then placed into amber-colored vials. Before conducting HPLC analysis, all samples were stored at 5°C in a dark environment. Samples were collected on multiple occasions until the concentrations of T1 and T2 decreased below the LOQ. After completing the analysis based on the occasions and concentrations, we calculated the half-life (DT50) of picloram using the provided formula.

The first-order kinetics form is:

$$-\ln \frac{Ct}{C0} = k$$

$$t1/2 = DT50 = \frac{\ln 2}{k} = \frac{0.6931}{k}$$

Where,
C0 - Herbicide concentration at time 0
Ct - Herbicide concentration at time t,
k - The rate constant.

Sample process

The representative amalgamated 5 mL from each concentration of water samples, concentrating them to dryness through a vacuum rotary evaporator, and subsequently diluted with acetonitrile to the designated mark. The prepared solution was then injected into the HPLC.

RESULTS AND DISCUSSION

Specificity

Specificity was established by the injection of Mobile phase solvents, specifically acetonitrile and 0.1% orthophosphoric acid, along with the sample solution, standard solution, and Lake Water. The chromatograms did not reveal any matrix peaks that would interfere with the analysis of picloram residues, as depicted in Figure 1. Moreover, the retention time for picloram was consistently recorded at 3.9 ± 0.2 min.

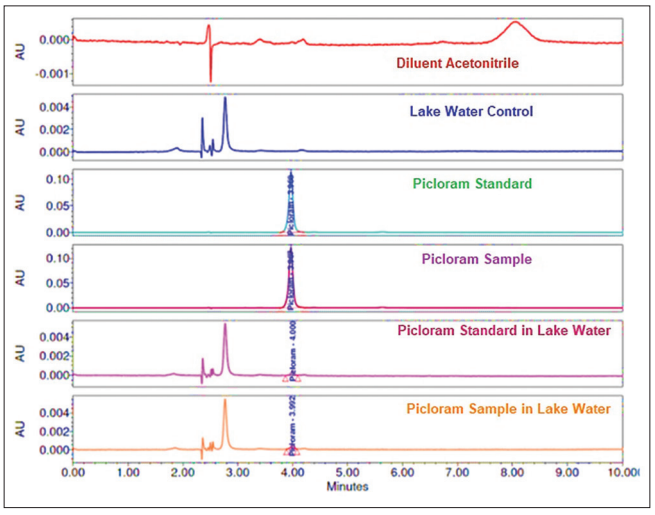

Figure 1: Representative specificity chromatogram of picloram

Table 1: Serial dilutions for linearity standard solutions and their response

Standard stock (µg/mL)	Volume taken (mL)	Final volume (mL)	Final concentration (µg/mL)	Standard area
1.00	0.150	10	0.015	246
1.00	0.500	10	0.05	874
100.07	0.100	10	1.00	16524
1000.74	0.100	10	10.01	168541
1000.74	0.500	10	50.04	800254
1000.74	1.000	10	100.07	1629022

Linearity

Different known concentrations of fungicides (0.015, 0.5, 1.0, 10.0, 50.0, and 100.0 µg/mL) were prepared in distinct 10 mL volumetric flasks by diluting the stock solution. The details of the serial dilution and responses were provided in Table 1. These standard solutions were injected directly into an HPLC. The representative chromatograms are shown in Figure 2. A calibration curve was plotted to show the relationship between the concentration of the injected standards and the observed area and the linearity of the method was evaluated by analyzing six standard concentration solutions. The peak areas obtained from the different concentrations of standards were used to calculate the linear regression equation, which is Y = 16225.93× + 16.37, with a correlation coefficient of 0.9999. A calibration curve is shown in Figure 3.

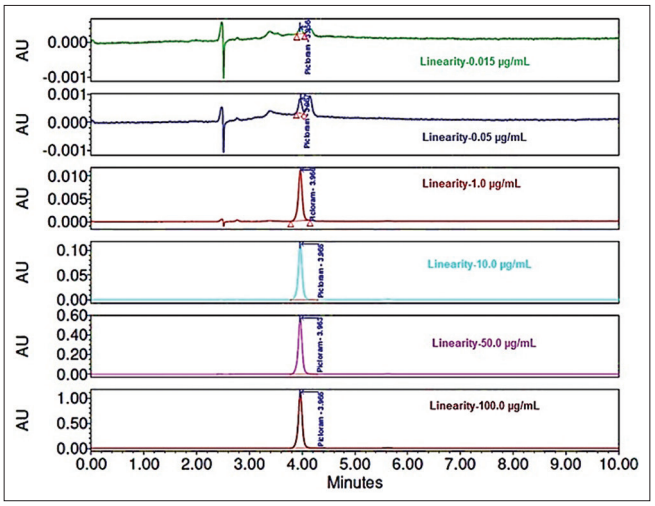

Figure 2: Representative linearity chromatograms of picloram

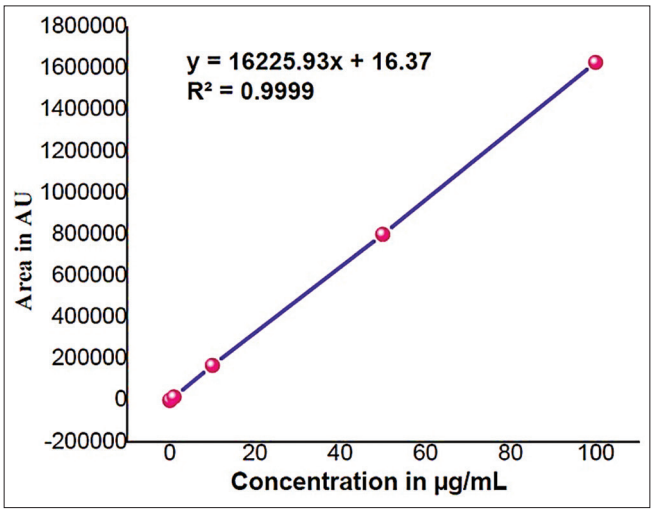

Figure 3: Calibration curve of picloram standard

Recovery and repeatability

The analytical method was validated for the recovery of the test item at three concentration levels with acidic, neutral, and basic water.

Preparation of test item stock solution

Accurately 5.17 mg of test item (purity 24.12%) of picloram was taken into a 50 mL volumetric flask. The content was dissolved in 20 mL of acetonitrile, sonicated, and made up to the mark with the same solvent. This concentration was a 24.94 µg/mL solution.

Preparation of 0.05 µg/mL fortification level

A 20 µL aliquot of 24.94 µg/mL test item stock solution was fortified into 10 mL of lake water. This was done in 6 replications.

Preparation of 0.5 µg/mL fortification level

A 200 µL aliquot of 24.94 µg/mL test item stock solution was fortified into 10 mL of lake water. This was done in 6 replications.

Preparation of 2.5 µg/mL fortification level

1.0 mL aliquot of 24.94 µg/mL test item stock solution was fortified into 10 mL of lake water. This was done in 6 replications.

The samples were assayed for accuracy and repeatability in HPLC. Accuracy was calculated as % recovery and repeatability as % relative standard deviation and the results

are mentioned in Table 2. The representative chromatograms are shown in Figure 4.

Detection and quantification limits

The LOQ has been set at 0.05 µg/mL. It is defined as the lowest fortification level tested that yields acceptable average recoveries (82–87%, RSD <2%). In addition, this quantification limit signifies the fortification level at which an analyte peak is consistently observed at approximately 10 times the baseline noise in the chromatogram. The LOD was identified as 0.015 µg/mL, which corresponds to a level of around 3 times the background of the control injection at the retention time of the peak of interest.

Table 2: Recovery and repeatability of picloram 24% SL

Fortification concentration in µg/mL	Replication	Recovery % and repeatability in lake water
0.05	R1	82.24
	R2	84.17
	R3	81.65
	R4	84.31
	R5	83.74
	R6	86.07
	Mean	83.70
	SD	1.58
	RSD	1.89
0.5	R1	89.79
	R2	91.26
	R3	92.45
	R4	90.63
	R5	92.37
	R6	90.85
	Mean	91.23
	SD	1.04
	RSD	1.14
2.5	R1	95.22
	R2	95.78
	R3	96.05
	R4	97.14
	R5	96.39
	R6	95.58
	Mean	96.03
	SD	0.68
	RSD	0.70

RSD: Relative standard deviation, SD: Standard deviation, SL: Soluble liquid

Dissipation details of picloram in lake water

The initial concentration of picloram in acidic water (day 0) was 0.952 µg/mL and 1.895 µg/mL in T1 and T2 dosages, respectively. The representative chromatograms presented in Figure 5, which on day 1 dissipated to 0.819 µg/mL and 1.626 µg/mL. The day 3 samples showed the residues 0.623 µg/mL (T1) and 1.348 µg/mL (T2), day 5 samples showed 0.517 µg/mL (T1) and 1.121 µg/mL (T2), day 10 samples showed 0.312 µg/mL (T1) and 0.662 µg/mL (T2), and day 20 samples showed 0.113 µg/mL (T1) and 0.227 µg/mL (T2). A complete dissipation of residues to below the detectable level was observed on day 30 in both the tested dosages (T1) and (T2). The representative chromatograms are presented in Figure 6.

The dissipation curve plotted between the concentration of the analyte and sampling occasions is presented in Figure 7 DT50 was calculated using the following formula.

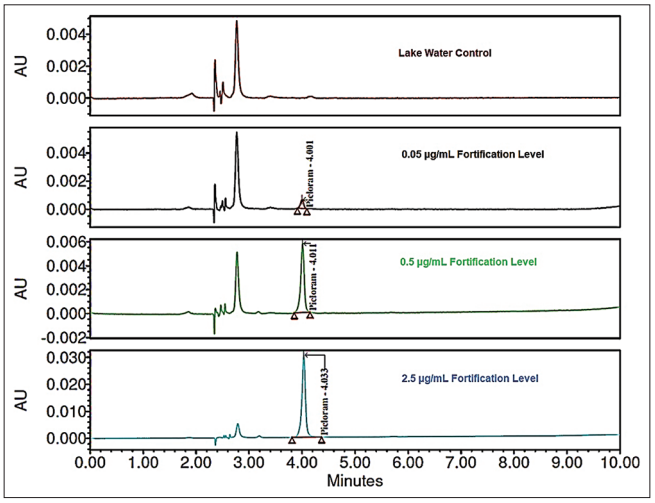

Figure 4: Representative recovery and repeatability chromatograms of picloram 24% soluble liquid

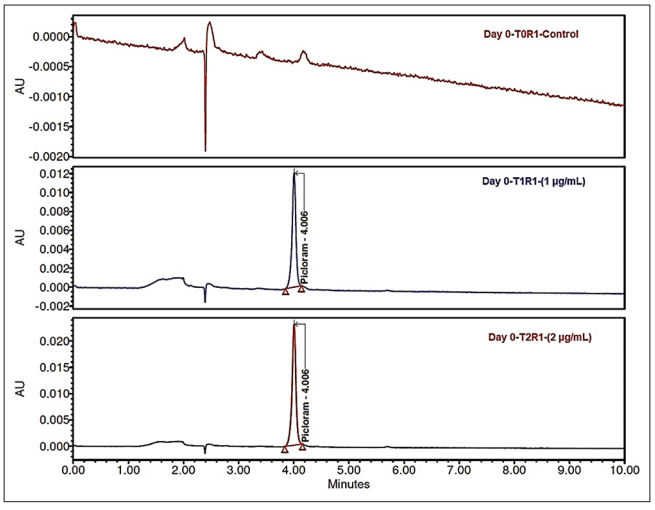

Figure 5: Representative day 0 occasion chromatograms of picloram in lake water

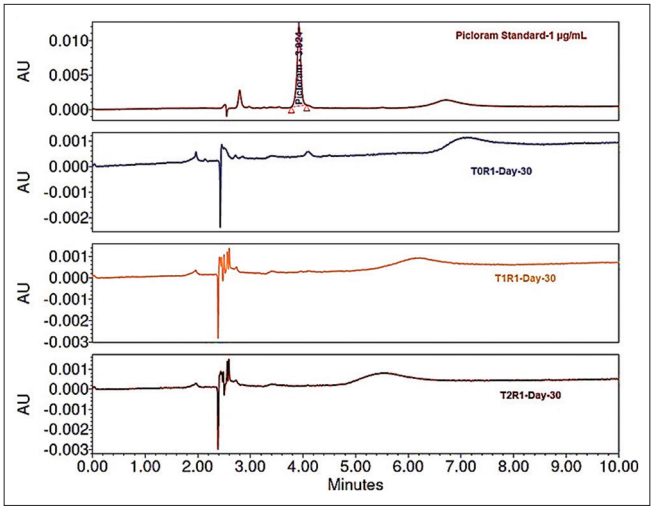

Figure 6: Representative day 30 occasion chromatograms of picloram in lake water

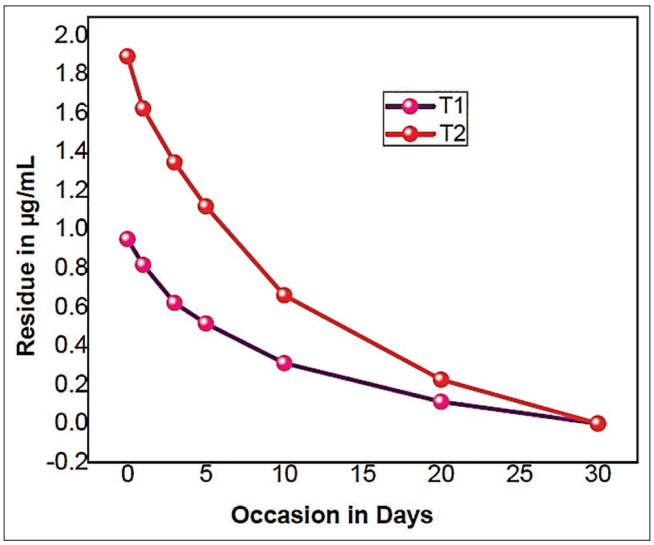

Figure 7: Dissipation curve of picloram in lake water

$$DT50 = \ln 2/(k)$$

Where,

"k" is the slope of the curve obtained from the dissipation data.

The calculated DT 50 (Time required to degrade 50% of residues) values are presented in Table 3. The rate constant value was calculated by a linear regression equation from the first-order rate equation.

$$K = \ln a/a\text{-}x/dt$$

Where, dt is the time interval between t_1 and t_2 and a, x is the concentration of pesticides at times t_1 and t_2, respectively. A plot of concentration of the residues and rate with the R^2 indicates first-order kinetics in the dissipation of both the fungicides. The DT50 (Half Life) of picloram was calculated by regression analysis from the dissipation data. The results were presented in Table 3.

Table 3: Regression analysis of picloram in lake water

T1			T2		
Days	**Conc.**	**Log**	**Days**	**Conc.**	**Log**
0	0.952	−0.0214	0	1.895	0.2776
1	0.819	−0.0867	1	1.626	0.2111
3	0.623	−0.2055	3	1.348	0.1297
5	0.517	−0.2865	5	1.121	0.0496
10	0.312	−0.5058	10	0.662	−0.1791
20	0.113	−0.9469	20	0.227	−0.6440
30	BDL	BDL	30	BDL	BDL
Slope		−0.0454	Slope		−0.0456
Intercept		−0.0468	Intercept		0.2703
Correl		−0.9988	Correl		−0.9997
DT50		6.63	DT50		6.60

BDL: Below the detectable level

CONCLUSION

This study details a rapid and efficient analytical method using HPLC-PDA to quantify picloram residues in three types of buffers. The mobile phase, comprising acetonitrile and 0.1% ortho-phosphoric acid, achieved good separation and resolution, with a very short analysis time of about 10 min for each chromatographic run.

Validation parameters, including linearity, recovery, precision, and LOQ, along with DT 50 values, were established satisfactorily by following the guidelines of the South African National Civic Organization and the Environmental Protection Agency. Therefore, the proposed analytical method and dissipation data could be advantageous for routine monitoring, residue laboratories, and researchers in assessing picloram residues in various commodities such as crops, water, and soil samples. The study also investigates the dissipation kinetics of the picloram herbicide in lake water, evaluating its environmental effects and persistence in aquatic ecosystems. The results indicate that the DT50 (Half-Life) of picloram is 6.63 days for the T1 dose and 6.60 days for the T2 dose.

REFERENCES

1. Imfeld G, Payraudeau S, Tournebize J, Sauvage S, Macary F, Chaumont C, *et al*. The role of ponds in pesticide dissipation at the agricultural catchment scale: A critical review. Water 2021;13:1202.
2. Székács A, Mörtl M, Darvas B. Monitoring pesticide residues in surface and ground water in Hungary:

Surveys in 1990-2015. J Chem 2015;2015:1-15.
3. Vallée R, Dousset S, Billet D. Influence of substrate water saturation on pesticide dissipation in constructed wetlands. Environ Sci Pollut Res 2015;23:109-19.
4. Syafrudin M, Kristanti RA, Yuniarto A, Hadibarata T, Rhee J, Al-Onazi WA, *et al*. Pesticides in drinking water-a review. Int J Environ Res Public Health 2021;18:468.
5. Droz B, Drouin G, Maurer L, Villette C, Payraudeau S, Imfeld G. Phase transfer and biodegradation of pesticides in water-sediment systems explored by compound-specific isotope analysis and conceptual modeling. Environ Sci Technol 2021;55:4720-8.
6. Vryzas Z. Pesticide fate in soil-sediment-water environment in relation to contamination preventing actions. Curr Opin Environ Sci Health 2018;4:5-9.
7. Yang SH, Choi H. Insecticides chlorantraniliprole and flubendiamide in *Aster scaber*: Dissipation kinetics, processing effects, and risk assessment. Heliyon 2024;10:e33216.
8. Sankhla MS, Kumari M, Sharma K, Kushwah RS, Kumar R. Water contamination through pesticide and their toxic effect on human health. Int J Res Appl Sci Eng Technol 2018;6:967-70.
9. Dhiman A, Toshikhane H. An analytical study of washing pesticides on cauliflower using traditional methods. J Ayurveda 2022;16:34-9.
10. Rao TN, Prashanthi Y, Manohranaidu T. Nanoparticles for Photocatalytic Remediation of Pesticide Residues in Environmental Samples. Vol. 3. Karnataka: IIP Series; 2024. p. 215-28.
11. Ahmed F, Rao TN, Arshi N, Prashanthi Y, Kumar S, Alshoaibi A. Fe_2O_3-Ag_2O/TiO_2 nanocatalyst-assisted LC-MS/MS-based detoxification of pesticide residues in daphnia magna and algae mediums. Crystals 2023;13:644.
12. Nageswara Rao T, Prashanthi Y, Ahmed F, Kumar S, Arshi N, Rajasekhar Reddy G, *et al*. Photocatalytic applications of Fe-Ag Co-doped TiO_2 nanoparticles in removal of flumioxazin pesticide residues in water. Front Nanotechnol 2021;3:652364.
13. Rao TN, Gaikwad S, Naidu TM, Han S. Extraction and determination of pesticide residues in water using carbon nanotubes coupled with gas chromatography-mass spectroscopy. Korean J Chem Eng 2020;37:1042-9.
14. Raju R, Rao TN. Bentazone herbicide residues from water. Asian J Pharm 2024;18:920-28.
15. Daqa WM, Alshoaibi A, Ahmed F, Rao TN. Potential applications of chitosan-coated zinc oxide nanoparticles for degrading pesticide residues in environmental soils. Crystals 2023;13:391.
16. Mwakalesi AJ, Potter ID. Removal of picloram herbicide from an aqueous environment using polymer inclusion membranes. J Environ Chem Eng 2020;8:103936.
17. Santilio A, Girolimetti S, Picardo V. Simple and rapid high-performance liquid chromatography method for simultaneous determination of picloram and 2,4-D in pesticide formulations. Analytica 2022;3:430-8.
18. Zhao P, Wang L, Chen L, Pan C. Residue dynamics of clopyralid and picloram in rape plant rapeseed and field soil. Bull Environ Contam Toxicol 2011;86:78-82.
19. Tian Y, Liu X, Dong F, Xu J, Lu C, Kong Z, *et al*. Simultaneous determination of aminopyralid, clopyralid, and picloram residues in vegetables and fruits using ultra-performance liquid chromatography/tandem mass spectrometry. J AOAC Int 2012;95:554-60.
20. Swathi R, Ramanjulu C, Ramachandra B, Naidu NV. Development and validation of spectrophotometric method for the determination of picloram. Der Pharm Lettre 2015;7:86-93.
21. Kim IS, Shim JH, Suh YT, Yau KY, Hall JC, Trevors JT, *et al*. Green fluorescent protein-labeled recombinant fluobody for detecting the picloram herbicide. Biosci Biotechnol Biochem 2002;66:1148-51.
22. Assis EC, Silva AA, Barbosa LC, LR de Queiroz ME. Optimization and validation of the solid-liquid extraction technique for determination of picloram in soils by high performance liquid chromatography. Planta Daninha 2011; 29(3):683-96
23. Salazar JE, Sandoval OL, Hurtado DA. Analysis of the properties of picloram and proposal of a compound as its replacement. Int J Chem Tech Res 2016;9:192-201.

Source of Support: Nil. **Conflicts of Interest:** None declared.