

Quantification of the organophosphorus pesticide profenofos in soils by Thin Layer Chromatography using image processing in *Image J* software

Elvis Gilmar Gonzales-Condori, D.Sc.¹, Kathia Abigail Montalvo-Bernal, B.Sc.², Rosa Alvarez-Gonzales, Mtr.², Jonathan Gonzales-Condori, B.Sc.³

¹Universidad Tecnológica del Perú (UTP), Av. Tacna y Arica 160, Arequipa, Perú, elvgonzalesc@gmail.com

²Centro de Investigación Kodama S.A.C., Urb. Leoncio Prado U10, Paucarpata, Arequipa, Perú.

abigail_23_1994@hotmail.com, rosalizethag@gmail.com

³Universidad Católica de Santa María (UCSM), Urb. San José s/n Umacollo, Arequipa, Perú. jogonzaco@gmail.com

Abstract: Pesticide residuals in agricultural soils are a global problem that puts non-target organisms, including humans, at risk. The involvement of universities in agricultural activities can be beneficial for monitoring the contamination or residuality of pesticides used on farmland. Therefore, in the present investigation, a method was developed for the quantification of profenofos (PFF) by Thin Layer Chromatography using a mobile phase chloroform: acetone (60:40) obtaining a retention factor (R_f) of 0.35. Using image processing with Image J software resulted in a linear method with an R^2 completion coefficient of 0.9966 which is greater than 0.995, precise with a coefficient of variation (CV) of 1.78 % which is less than 2.7 %, accurate with a recovery percentage of 101.04 % which is in the range of 90 to 110 % with limits of detection and quantification of 0.0639 μg and 0.1522 μg respectively. PFF was quantified in onion and garlic agricultural soils, resulting in concentrations of 0.252 and 0.264 mg/kg, respectively. In conclusion, the present methodology could be adapted to determine other pesticides or be applied in the field for monitoring PFF in agricultural soils by universities or governmental entities, which could lead to more responsible agriculture.

Keywords. Profenofos, Image J, soils, Thin Layer Chromatography.

I. INTRODUCTION

The indiscriminate use of insecticides has caused serious environmental problems. Pesticides containing organophosphorus compounds are used more frequently in agricultural activities, and their use has been increasing due to the presence of multiple pests, which threaten the production of food of plant origin. [1]. The problem lies in their residual nature since these toxics can produce undesirable effects in the ecosystems near the croplands [2]. It is also dangerous for farmers, women being more sensitive than men. It is also known that children, pregnant women, the elderly, and sick people with altered metabolic pathways are especially sensitive to pesticide activity [3]. Because of this, the European Commission set two targets corresponding to the reduction of pesticides as part of its strategy with a 50% reduction in the use and risk of chemical pesticides, and a 50% reduction in the use of the most hazardous pesticides by 2030[4]. In Arequipa, Peru, the use of pesticides has been

increasing in recent years; therefore, it is necessary to monitor their concentration or residuality in soils to look for mitigation alternatives.

Among the pesticides of interest are organophosphorus compounds whose structure includes trivalent and pentavalent phosphorus, these chemicals are often considered insecticides or nerve agents whose acute toxicity arises from the inhibition of acetylcholinesterase (AChE) [5]. For this reason, the mismanagement or incorrect handling of these organophosphates is responsible for a large number of acute intoxications characterized by the development of cholinergic syndrome and multiple chronic complications, with delayed neuropathy being one of the most representative ones [6]. Among the organophosphorus compounds is profenofos (PFF), which is commonly used in Arequipa, Peru on garlic and onion crops. In the world, the use of PFF (Fig. 1) has increased due to its efficacy against pests resistant to other organophosphates, but its presence in the environment could produce acute or chronic intoxications, either occupational or by contact with contaminated soil, water, air or food [3], in addition, this organophosphate is one of the main toxicants present in fresh water and poses a significant risk to the health of fish [7].

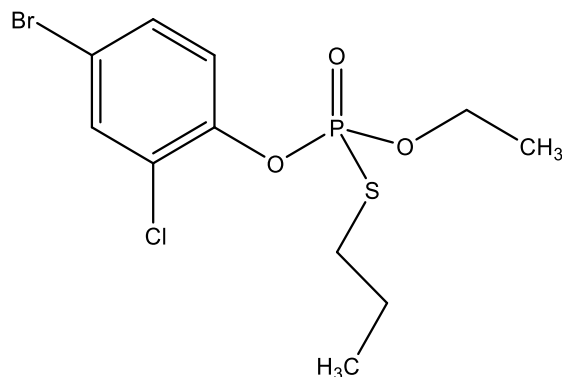


Fig. 1. Chemical structure of profenofos

Methods for the quantification of PFF using High Performance Liquid Chromatography (HPLC) have been

developed in the literature (HPLC) [8], [9], spectrophotometry based on nanotechnology [10] and gas chromatography with extraction by the QuEChERS method [11]. These methods are characterized by being the most used, however, the costs for their implementation in a laboratory are high, likewise, the analyses are also expensive making it almost impossible to develop research. Therefore, it is necessary to develop research on the monitoring of PFF that is economical and fast and that facilitates the acquisition of information on the concentration of this toxics in the environment and in this way, measure its possible impacts in this way government entities can better control farmers for the benefit of more environmentally friendly agriculture. For this reason, the present research aims at the quantification of the organophosphorus pesticide PFF in soils by Thin Layer Chromatography (TLC) through image processing in Image J software.

II. MATERIALS AND METHODS

A. Reagents and chromatographic conditions

In the present investigation solvents were used; acetone p.a. of Merck, and chloroform J.T. Baker. Silica gel F₂₅₄ plates were used, the chromatographic runs were carried out in a chromatographic tank, and a 2 % silver nitrate solution was used as a developer. Also, a profenofos Fluka standard of purity greater than 99.5 % obtained from Sigma Aldrich was used.

B. Extraction and identification of profenofos in soils

Fig. 2 shows the procedure for the extraction of PFF from the soil by an Ultrasound Assisted Solid to Liquid Extraction Method. It consists of weighing a sample of 5 grams of soil in a syringe conditioned with two filter papers, 2 grams of anhydrous sodium sulfate, then adding ethyl acetate (10 mL) and submitting it to ultrasound for 15 minutes, followed by gravity filtration in a test tube and a second extraction with 5 mL of ethyl acetate, then, the solution with extracted PFF was concentrated using nitrogen (N_{2(g)}) until dryness [1, 12, 13] and reconstituted with 100 µL ethyl acetate which is seeded on Merck F₂₅₄ silica gel plates [14]. Subsequently, the plate was taken to a chromatographic vat with the mobile phase of chloroform: acetone (60:40) which allowed the PFF to rise. Finally, the plate was dried and developed with 2 % silver nitrate and dried at 130 °C.

C. Image processing in Image J

The data and image processing was performed in *Image J* software which consists of processing the plate and adjusting the color with the “*Brightness/contrast*” command, followed by the “*Plot/lanes*” command resulting in peaks that represent an integrated baseline facilitating the reading of the areas. The areas processed with the “*Wand (Tracing) tool*” result in a numerical value (area in pixels) that is directly proportional to the size of the spot [14].

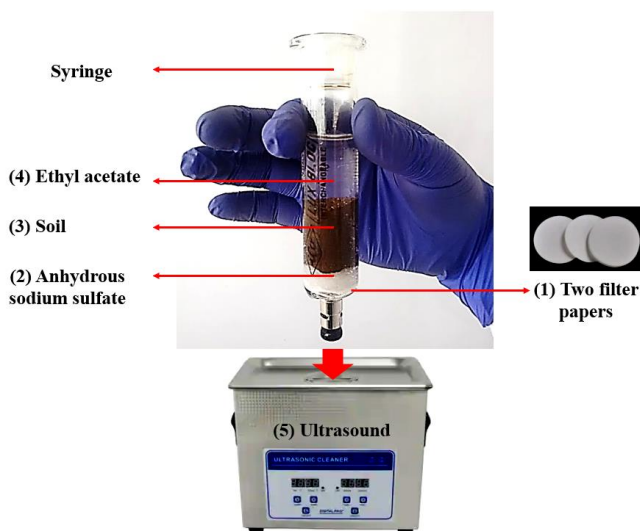


Fig. 2. Organophosphate extraction system. 1) filter paper, 2) anhydrous sodium sulfate, 3) soil, 4) ethyl acetate, 5) ultrasound.

D. Method validation

For the validation of the method, the retention factor was first identified in TLC using the mobile phase of chloroform: acetone (60:40). Subsequently, the validation parameters of linearity, sensitivity, precision, and accuracy were evaluated. This was done using the procedures detailed in a previous study [14]. For the precision and accuracy tests, synthetic soils contaminated with PFF were prepared as follows [1], [13].

E. Application in real agricultural soils

The methodology was applied to agricultural soils. From a cultivated field, 3 kg were taken from five points which correspond to four extreme points and one middle point. The 15 kg were collected and sieved in the same field to eliminate stones and larger particles. The soil was mixed until homogeneity using a shovel and 3 kg were taken to the laboratory. In the laboratory, another sieving was carried out with a 40 mesh, and sampling by quartering was done, taking 5 g for each extraction experiment.

III. RESULTS AND DISCUSSION

A. Identification of profenofos by Thin Layer Chromatography

The identification of PFF using as a developer an aqueous solution of AgNO₃ and a temperature of 130 °C results in a yellow to orange coloration (Fig. 3). In a previous study, the organophosphorus pesticides chlorpyrifos and methamidophos were identified in the same way, giving orange and yellow stains, respectively [14].

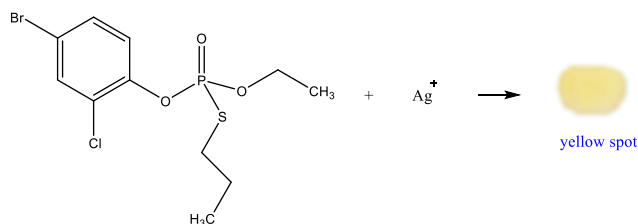


Fig. 3. Color reaction between the profenofos molecule and silver cations.

Using the chloroform: acetone (60:40) mobile phase, the PFF standard was found to run through the silica gel plate to an R_f of 0.35 as shown in Fig. 4. A previous study found that using chloroform: cyclohexane (60:40) mobile phase, chlorpyrifos had an R_f of 0.82 and with chloroform: acetone (80:20) mobile phase, methamidophos had an R_f of 0.19 [14].

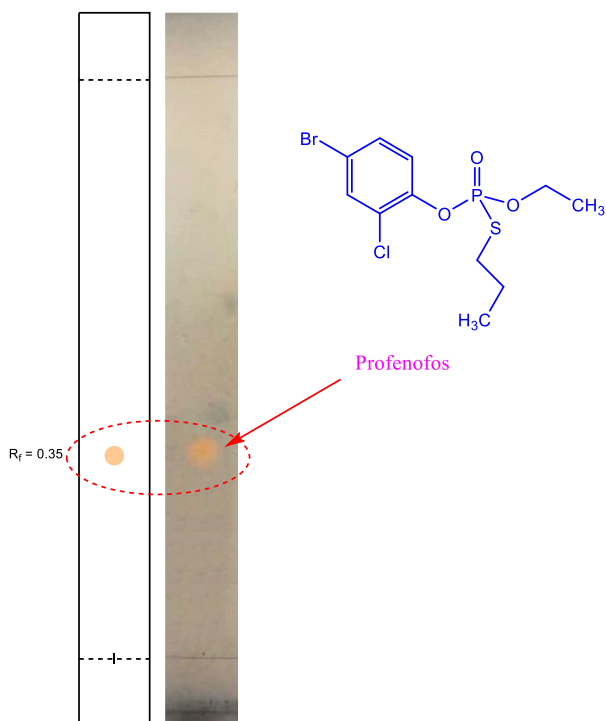


Fig. 4. Plate development with 2 % AgNO_3 after profenofos chromatographic run using as mobile phase chloroform: acetone in ratio (60:40).

B. Validation of the method to quantify profenofos by thin layer chromatography

Linearity

Fig. 5 shows the areas obtained after seeding in a silica gel plate volume of 5, 25, 50, 75, 125, and 175 μL of a standard PFF solution of 20000 mg/L, this is equivalent to an amount of 0.1, 0.5, 1.0, 1.5, 2.5 and 3.5 μg of PFF seeded. It is observed that the size of the spots increases as a function of

the amount of PFF seeded, likewise, the areas obtained in the Image J software expressed in pixels are presented.

Table I shows the areas in triplicate, the average area, and the standard deviation. It is observed that as the amount of profenofos planted is greater, the area increases.

TABLE I

AVERAGE AREAS AND STANDARD DEVIATION CONCERNING THE AMOUNT OF PROFENOFOS SEEDED ON TLC PLATES.

PFF (μg)	Area 1 (Píxeles)	Area 2 (Píxeles)	Area 3 (Píxeles)	Area Average (Píxeles)	Standard deviation
0.1	2290.77	2023.77	2391.73	2235.42	190.12
0.5	11735.01	12827.39	11699.10	12087.17	641.30
1.0	23527.66	20391.93	23982.11	22633.89	1954.86
1.5	39094.48	36214.82	34192.05	36500.45	2463.66
2.5	55652.53	57141.91	58212.19	57002.21	1285.54
3.5	72779.02	78228.60	76102.49	75703.37	2746.62

Plotting the amount of PFF (μg) versus the area in pixels in Table I, the calibration graph presented in Fig. 6 shows the linear equation and the coefficient of determination $R^2 = 0.9966$, which indicates that the method complies with the linearity parameter ($R^2 > 0.995$) [15]. The linear equation obtained corresponds to Equation 1:

$$y = 21739x + 1389.9 \quad (1)$$

Where “y” corresponds to the area in pixels and “x” to the amount of PFF in μg . Replacing these variables in the linear equation results in Equation 2:

$$\text{Area} = 21739[\text{PFF}_{\mu\text{g}}] + 1389.9 \quad (2)$$

Equation 3 is obtained by clearing PFF to calculate the μg of PFF in the samples.

$$\text{PFF}_{\mu\text{g}} = \frac{\text{Area} - 1389.9}{21739} \quad (3)$$

Sensitivity

For the determination of sensitivity, we first plotted the amount of PFF (μg) versus the area in pixels obtained from Table I. The graph is presented in Fig. 7 where the linear equation of that graph is also presented and is presented in Equation 4.

$$y = 577.36x + 671.35 \quad (4)$$

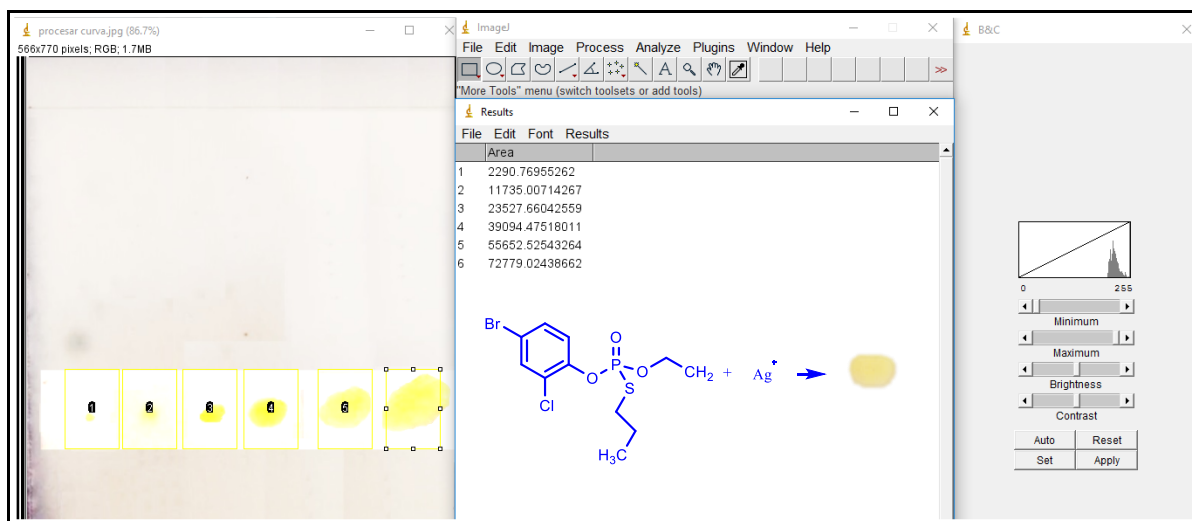


Fig. 5. Image processing using Image J software of the profenofos stains for calibration plotting

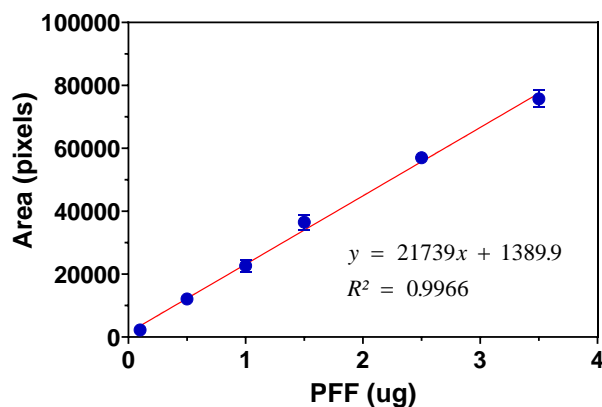


Fig. 6. Calibration plot relating the amount of profenofos in micrograms versus the area in pixels of the spots measured using Image J.

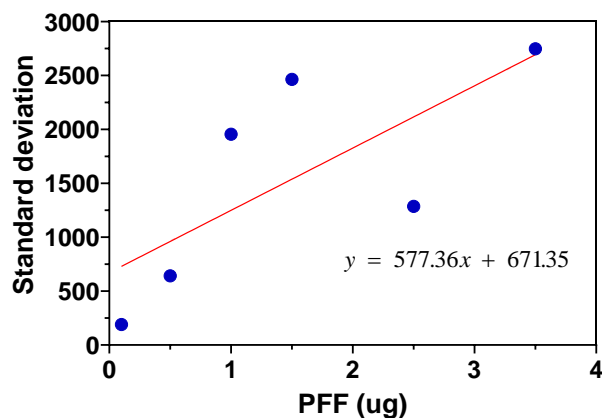


Fig. 7. Graph relating the amount of profenofos (PFF) in micrograms vs. the standard deviation of the PFF spots to calculate the sensitivity of the method.

Sensitivity was determined by calculating the limits of detection (LoD) and limits of quantification (LoQ), which are presented in Equation 5 and Equation 6, respectively.

$$LoD = \frac{Y_{bl} + 3(S_{bl})}{b} \cdot \frac{1}{\sqrt{n}} \quad (5)$$

$$LoQ = \frac{Y_{bl} + 10(S_{bl})}{b} \cdot \frac{1}{\sqrt{n}} \quad (6)$$

Where “ b ” corresponds to the slope obtained in Equation 1, “ Y_{bl} ” corresponds to the response of the blank “ Y_{bl} ” and is equivalent to the intercept of Equation 1. “ S_{bl} ” corresponds to the standard deviation of the blank and its value is the intercept of Equation 4. “ n ” corresponds to the number of points used for the linear equation (Table I) ($n=6$). Replacing the respective values in Equations 5 and 6 gives the limits of detection and quantification of the method as $0.0639 \mu\text{g}$ and $0.1522 \mu\text{g}$ respectively.

Precision

For the determination of the precision of the method, contaminated soils (10 g) were prepared with PFF in a synthetic form which should contain $5 \mu\text{g}$ of the organophosphate. PFF was extracted from 5 g soil according to the proposed methodology. The solvent was seeded with approximately $2.5 \mu\text{g}$ of extracted PFF on TLC plates and developed. The procedure was performed six times. The obtained spots were analyzed in *Image J* software.

Table II shows the results of the determination of the area of each spot in pixels, as well as the average, standard deviation, and coefficient of variation (CV (%)). The CV (%) was calculated with Equation 7 resulting in a value of 1.78 %. As it is less than 2 %, the method can be considered accurate [15].

$$CV(\%) = \frac{s}{\bar{x}} \times 100 \quad (7)$$

Where “s” and “ \bar{x} ” correspond to the standard deviation and average, respectively.

TABLE II

COEFFICIENT OF PERCENTAGE VARIATION (CV%) OF THE AREAS IN PROFENOFOS PIXELS PRESENT IN A SAMPLE OF SYNTHETIC SOIL ANALYZED BY TLC.

N	Area (Pixels)
1	55652.53
2	57141.91
3	58212.19
4	56727.34
5	57891.96
6	55998.89
Average	56937.47
Standard deviation	1015.28
CV (%)	1.78

Accuracy

Accuracy was expressed in terms of percent recovery (%R). For the determination of the accuracy of the method, the extraction of PFF from the remaining 5 g of soils analyzed in the precision test was initiated. The 5 g of soil were taken to syringes where they were spiked with 0.5 µg of standard PFF. The extraction was completed and the final solutions were seeded on TLC plates and then developed. Finally, the areas of the extractions were measured in *Image J* software.

Table 5 shows the results obtained from the sensitivity test, column (1) presents the areas obtained in the precision test that correspond to the areas in pixels found in the soils synthetically contaminated with profenofos, column (2) corresponds to the amount of PFF in these soils (S) that was calculated with Equation 3, column (3) corresponds to the amount of PFF with which the soils were enriched (E), column (4) presents the areas in pixels of the spots obtained from the analysis of the enriched soils and column (5) presents the amount in µg of [S+E] calculated with Equation 3. In the same table, the %R obtained from the six repetitions of the procedure is the average of 101.04 %. This value is between 90 and 110 %, which would indicate that the method is accurate [15].

$$\%R = \frac{[S + E] - S}{E} \times 100 \quad (8)$$

In a study conducted by HPLC to quantify PFF by HPLC, the calibration graph prepared for the method of determination of PFF in soils at concentrations of 0.5 to 3.0 mg/L showed a coefficient of determination (R^2) of 0.9994, on the other hand, the coefficient of variation (CV%) was 2.27% and the

accuracy calculated by the percentage recovery method (%R) was 99.06 % [13]. In the present investigation, the validation parameters are also met using TLC.

TABLE III

DETERMINATION OF PERCENTAGE RECOVERY OF THE METHOD TO QUANTIFY PROFENOFOS BY CCF TO DETERMINE THE ACCURACY.

N	PFF					%R
	(1) Area (Píxeles) (S)	(2) Amount (µg) (S)	(3) Amount (µg) (E)	(4) Area (píxeles) (S + E)	(5) Amount (µg) (S + E)	
1	55652.53	2.49	0.5	67211.43	3.03	106.34
2	57141.91	2.57	0.5	68921.23	3.11	108.37
3	58212.19	2.61	0.5	69192.33	3.12	101.02
4	56727.34	2.55	0.5	66982.86	3.02	94.35
5	57891.96	2.59	0.5	68230.31	3.08	95.11
6	55998.89	2.51	0.5	66981.79	3.02	101.04
Average recovery percentage						101.04

C. Application in agricultural soils

Onion cultivation

Fig. 8 shows the result of the quantification of PFF in onion crop soils with the validated methodology, resulting in an area of 28744.23 pixels. Replacing it in Equation 3 gives an Amount of 1.258 µg. Subsequently, the PFF concentration in soils in mg/kg was calculated using Equation 9.

$$PFF(mg / kg) = \frac{PFF_{\mu g}}{m \times 1000} \quad (9)$$

Where “m” is the amount of soil analyzed in kg (0.005 kg) and 1000 is used for the conversion from µg to mg. Replacing it is obtained that the onion crop soil presents a concentration of 0.252 mg/kg of PFF.

Garlic cultivation

Fig. 9 shows the result of the quantification of PFF in garlic crop soils using the validated method using *Image J* resulting in an area of 30129.4975 pixels. Replacing the values in Equation 3 and Equation 9 resulted in a concentration of 0.264 mg/kg of PFF in garlic soil. A previous study quantified chlorpyrifos by TLC in garlic and onion soil yielded concentrations of 0.19 and 0.13 mg/kg, respectively, and methamidophos in alfalfa soil with 0.032 mg/kg [14]. Another study found chlorpyrifos and PFF in chili bell pepper (*Capsicum annuum*) soils at concentrations of 0.801 ± 0.072 mg/kg and 0.592 ± 0.017 mg/kg, respectively [12].

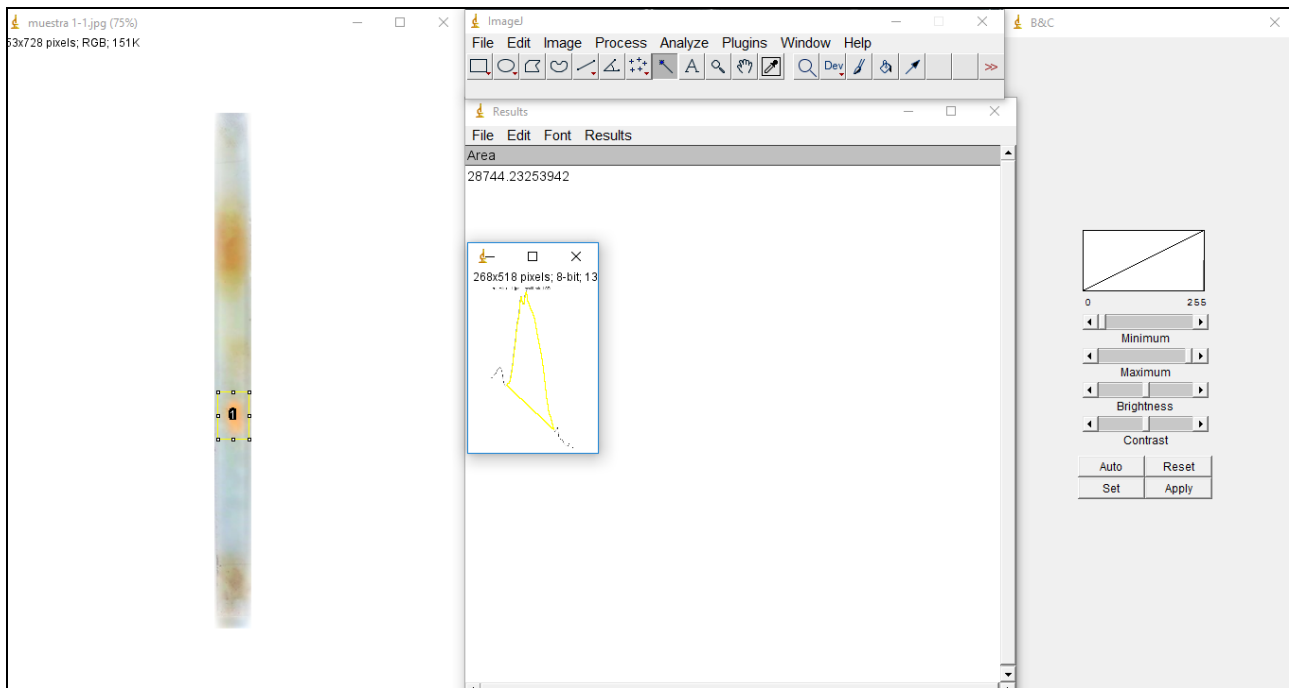


Fig. 8. Image processing for the determination of profenofos in onion crop soil using *Image J*.

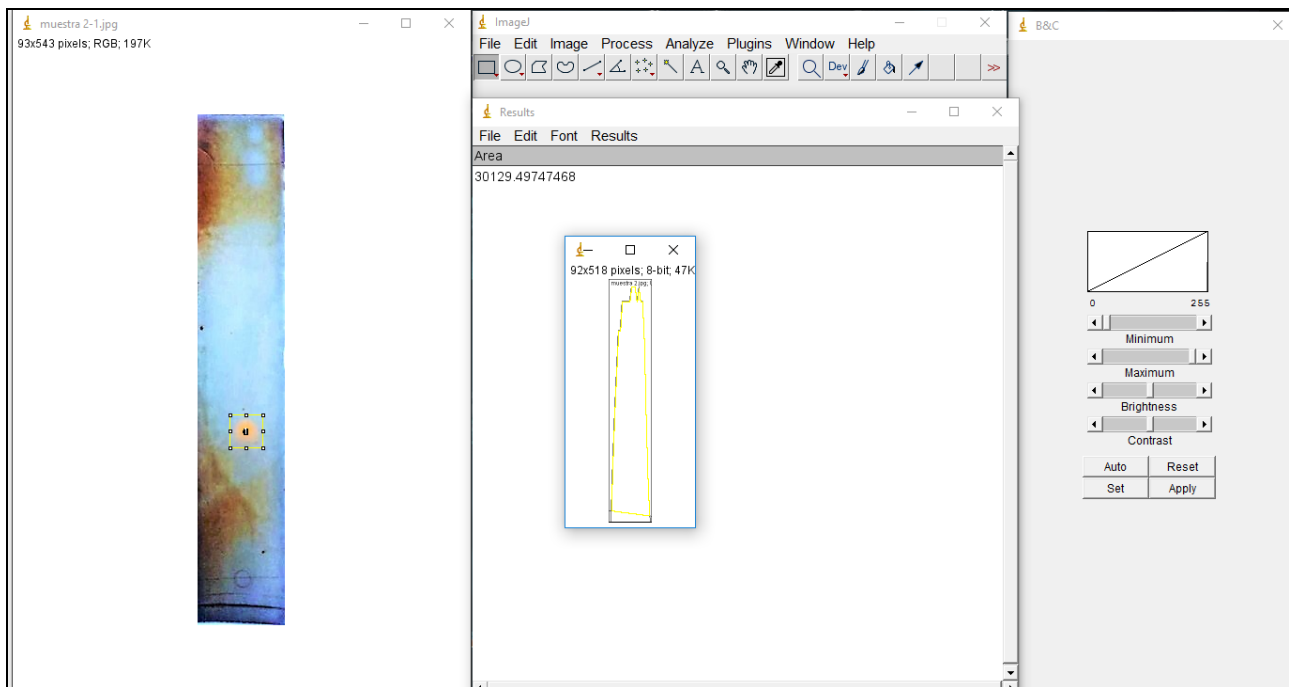


Fig. 9. Image processing for the determination of profenofos in garlic crop soil using *Image J*.

III. CONCLUSIONS

It was demonstrated that the method to extract and quantify profenofos in soils by TLC using Image J as image processing software is linear ($R^2= 0.9966$) which is greater than 0.995, precise ($CV\%= 1.78 \%$) and accurate ($\%R= 101.04 \%$) with detection and quantification limits of $0.0639 \mu\text{g}$ and $0.1522 \mu\text{g}$

respectively. It was possible to apply the methodology developed in agricultural soils where onion and garlic are grown, where PFF was found with a concentration of 0.252 and 0.264 mg/kg, respectively. This research could be considered by farmers in conjunction with universities that want to monitor PFF in soils using cost-effective resources.

REFERENCES

- [1] E. G. Gonzales-Condori, C. Choquenaira-Quispe, and S. A. Ramírez-Revilla, "Study of the degradation of chlorpyrifos in contaminated soils in the presence of the red californian earthworm *Eisenia foetida*," *Revista Internacional de Contaminación Ambiental*, vol. 36, no. 1, Art. no. 1, Jan. 2020, doi: 10.20937/RICA.2020.36.53201.
- [2] G. J. Devine, D. Eza, E. Ogusuku, and M. J. Furlong, "Uso de insecticidas: contexto y consecuencias ecológicas," *Revista Peruana de Medicina Experimental y Salud Publica*, vol. 25, no. 1, pp. 74–100, Jan. 2008.
- [3] A. M. Del Puerto Rodríguez, S. Suárez Tamayo, and D. E. Palacio Estrada, "Efectos de los plaguicidas sobre el ambiente y la salud," *Revista Cubana de Higiene y Epidemiología*, vol. 52, no. 3, pp. 372–387, Dec. 2014.
- [4] V. Silva, H. G. J. Mol, P. Zomer, M. Tienstra, C. J. Ritsema, and V. Geissen, "Pesticide residues in European agricultural soils – A hidden reality unfolded," *Science of The Total Environment*, vol. 653, pp. 1532–1545, Feb. 2019, doi: 10.1016/j.scitotenv.2018.10.441.
- [5] R. Richardson and G. Makhaeva, "Organophosphorus Compounds," in *Encyclopedia of Toxicology*, 2014, pp. 714–719. doi: 10.1016/B978-0-12-386454-3.00173-1.
- [6] E. Benedico, "Insecticidas organofosforados: 'De la guerra química al riesgo laboral y doméstico,'" *Medifam*, vol. 12, May 2002, doi: 10.4321/S1131-57682002000500005.
- [7] Zeinab. M. El-bouhy, Fatma. A. S. Mohamed, Mohamed. W. A. Elashhab, and W. El-Houseiny, "Toxicity bioassay and sub-lethal effects of profenofos-based insecticide on behavior, biochemical, hematological, and histopathological responses in Grass carp (*Ctenopharyngodon idella*)," *Ecotoxicology*, vol. 32, no. 2, pp. 196–210, 2023, doi: 10.1007/s10646-023-02628-9.
- [8] H. Naik, P. H Naik, P. Devanna, R. Haveri, and U. Nidoni, "Determination of Profenofos Residues using LC-MS/MS and Its Dissipation Kinetics in Pigeonpea Pods," *Legume Research - An International Journal*, Aug. 2020, doi: 10.18805/LR-4330.
- [9] M. Tamandani, S. H. Hashemi, M. Kaykhani, A. Jamali Keikha, and A. Nasiryan, "Determination of profenofos in seawater and foodstuff samples after its molecularly imprinted polymer pipette-tip micro solid phase extraction optimized by response surface methodology," *BMC Chemistry*, vol. 16, no. 1, p. 12, Mar. 2022, doi: 10.1186/s13065-022-00807-z.
- [10] Z. Lu, J. Li, J. Shen, and H. Wang, "Spectrophotometric Determination of Profenofos Using an Aptamer-Gold Nanorod (GNR)-Based Biosensor," *Analytical Letters*, vol. 56, no. 9, pp. 1514–1524, Jun. 2023, doi: 10.1080/00032719.2022.2135726.
- [11] G. Bellisai *et al.*, "Targeted review of maximum residue levels (MRLs) for profenofos," *EFSA J*, vol. 21, no. 12, p. e8445, Dec. 2023, doi: 10.2903/j.efsa.2023.8445.
- [12] E. G. Gonzales-Condori, G. Avalos-López, Y. Vargas-Alarcón, J. M. Medina-Pérez, J. A. Villanueva-Salas, and G. Briceño, "Simultaneous degradation of chlorpyrifos and profenofos in soils at sublethal concentrations in presence of *Eisenia foetida* and a native bacterial consortium," *Environmental Advances*, vol. 16, p. 100514, Jul. 2024, doi: 10.1016/j.envadv.2024.100514.
- [13] E. G. Gonzales-Condori, S. A. Ramírez-Revilla, and J. A. Villanueva-Salas, "Role of *Eisenia foetida* in the degradation of profenofos in presence of native bacterial communities," *Revista Mexicana de Ingeniería Química*, vol. 19, no. Sup. 1, Art. no. Sup. 1, Jun. 2020, doi: 10.24275/rmiq/IA1505.
- [14] E. G. Gonzales-Condori, J. C. Reynoso-Espinoza, R. Yucra-Condori, J. K. Ramirez, and C. Choquenaira-Quispe, "Desarrollo De Un Método Rápido Para La Cuantificación De Metamidofos Y Clorpirifós Por Cromatografía En Capa Fina En Suelos Mediante Procesamiento De Imágenes," Aug. 2022, Accessed: Oct. 07, 2022. [Online]. Available: <https://laccei.org/LACCEI2022-BocaRaton/meta/FP781.html>
- [15] O. Quattrocchi, S. Abelaira, and R. Felipe Laba, *Introducción a la HPLC, Aplicación y Práctica*. 1992.