

Brief Report

Contamination by the Herbicide 2,4-D in the Agro-Industrial Karst of Yucatán, México

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Abstract

Agribusiness is the main economic activity in northeastern Yucatán, México. The production of forage grasses for livestock feed requires weed control, achieved through chemical methods, with the herbicide 2,4-D being the most widely used in the region. This research quantified the presence of 2,4-D in groundwater in Sucilá, Yucatán, during 2023. Twenty ranches with different stocking rates (organisms/hectare) were evaluated. Groundwater samples and soil cores were collected in two climatic seasons (dry and rainy) to relate soil properties and herbicide concentration to the weather. The results showed that the concentration of 2,4-D was higher during the rainy season (September 2023), with values between 1.6 and 3.6 mg 2,4-D/L, but no differences were observed among stocking rates. Soil organic matter was different between seasons, and cation exchange capacity changed among stocking rates. Continuous irrigation, coupled with the application of agrochemicals, is likely impairing the water quality of groundwater in this area of the Yucatan.

Keywords: cation exchange capacity; groundwater contamination; irrigation; organic matter; Yucatán karst aquifer

1. Introduction

In the Northeast region of Yucatán, agricultural activity (farming and livestock) is the main economic activity, where the production of forage grass and induced vegetation forms the basis for feeding livestock [1]. Herbicides are considered the most effective method because they efficiently, quickly, and economically eliminate weeds over large areas [2,3]. In Mexico, estimates of herbicide use are around 44,000 on an annual basis [4]. In the state of Yucatán (east México), by 2005, pesticide consumption had increased to approximately 4800 tons [5]. Currently, the exact figure for herbicide use remains unknown. In 2022, Mexican agencies compiled a list of the most frequently used pesticides from 1980 to 2018, where 2,4-D ranked third in use across the 22 states studied, and second in application during the 2007–2013 period, with a total application of 273,239 kg a.i. (active ingredient) [6]. This herbicide is one of the most prevalent in the agro-industrial karst as a method of controlling broadleaf weeds in forage grass crops. 2,4-D is a synthetic auxin that inhibits plant growth and interferes with nucleic acid metabolism, leading to plant death [7]. It is one of the oldest and most widely used herbicides because of its selectivity for broadleaf weeds and harmlessness to grasses. It is highly soluble in



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water, but exhibits relatively low absorption in the soil, especially in soils with low organic matter content, resulting in leaching into groundwater [8]. Commercial 2,4-D formulations available as amine salts or esters are slightly acidic ($\text{pH} \approx 6$) and highly soluble (620 to 23,000 mg 2,4-D/L) [9], containing adjuvants that contribute to improving their effects on plants [10]. It has a relatively short half-life in soil, ranging from two to 13 days [11], but is not easily biodegradable in aqueous environments; 2,4-D esters may persist in acid aquatic environments and dry soils [12].

Groundwater contamination has been documented in Yucatán, associated with the use of agrochemicals that infiltrate and degrade water quality [13]. Using the groundwater ubiquity score index (GUS) derived from the physical properties of 2,4-D, it has been evaluated with a high degree of mobility [14]. It is the second most widely used herbicide in agro-industrial karst [15]. It has been reported that 2,4-D has health side effects when the application and handling guidelines were not followed [5]. It is moderately toxic to birds and mammals, slightly toxic to fish [16], and causes bee deaths and minor adverse effects in humans, ranging from eye irritation and headaches to death by ingestion [5,17]. While 2,4-D esters and amine salts can persist in aquatic environments and dry soils before hydrolyzing to 2,4-D acid [12], the degradation intermediates 2,4-dichlorophenol (2,4-DCP) and 3,5-dichlorocatechol (3,5-DCC) exhibit strong ecotoxic effects, with 3,5-DCC demonstrating higher toxicity than the parent compound [2,18]. Although it is not considered high-risk in Mexico, this product was added to the list of potentially hazardous pesticides in 2019 due to contamination during its production with a highly toxic by-product, 2,3,7,8-tetrachlorodibenzo-p-dioxin [19]. The list is based on classifications from International and recognized authorities, compiled from entities such as the World Health Organization (WHO), the European Commission, national agencies, and the US EPA. Updates are based on documented cases of pesticide active ingredients and formulations that have shown a high incidence of serious and irreversible adverse effects on human health or the environment, when robust, evidence-based, and publicly accessible data are available [19].

The hazard criteria are grouped into acute toxicity, long-term (chronic) health effects, environmental hazard criteria, and international regulations and global conventions related to pesticides. What type of pesticides have been included across the different years is addressed, but mainly the main criteria of inclusion in the list and how they are updated.

The objective of this research was to quantify the concentration of 2,4-D in cattle ranches in tropical karst landscapes, considering low and high stocking rates. Additionally, the potential risk of soil mobilization was explored by evaluating soil properties (organic matter, cation exchange capacity, and pH). The aim of this research was to assess whether the concentration of 2,4-D in irrigation water was influenced by stocking rates.

2. Materials and Methods

2.1. Study Area

This research was done in northeastern Yucatán, on 20 cattle ranches in the municipality of Sucilá (Figure 1 and Supplementary Table S1). This region is the least populated, with agricultural activities covering half of its surface area (49%), predominantly cultivated pastures and extensive cattle ranching [20]. The municipality has an average altitude of 16 m a.s.l., a warm sub-humid climate with summer rains, an average annual temperature of 26.4 °C, and an average annual rainfall of 800 mm [21]. At the regional level, the area is characterized by its low relief, with thin Leptosols, Histosols, Cambisols, Vertisols, and Gleysols [22,23].

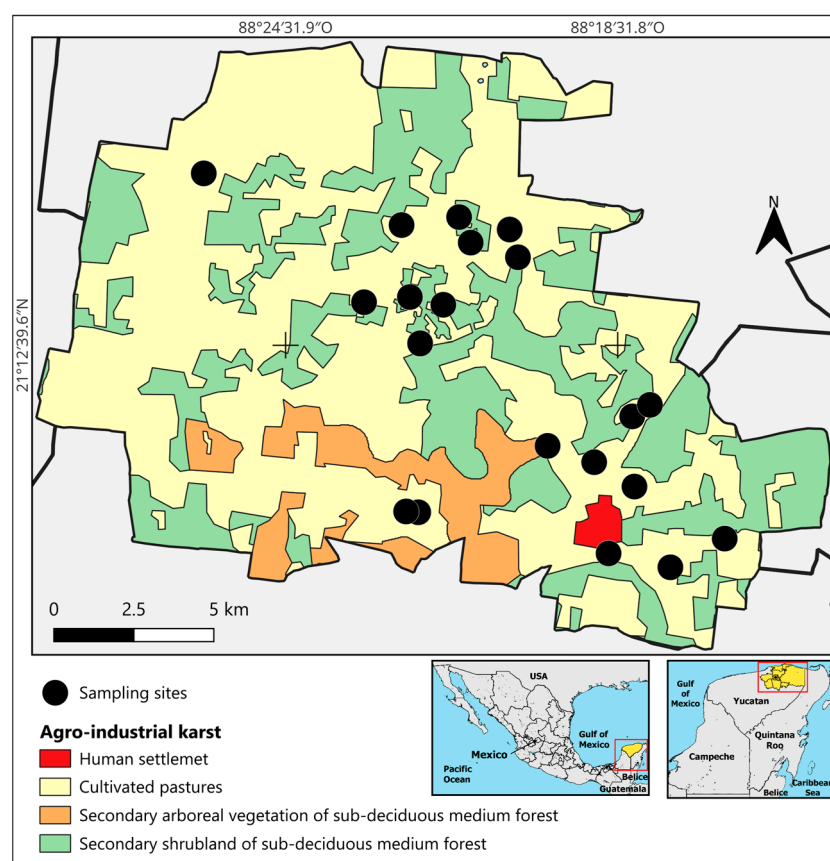


Figure 1. Location of the study sites in the agro-industrial karst zone of northeastern Yucatán, Mexico.

2.2. Selection Criteria

Sampling sites were selected based on interviews conducted with property owners or managers to obtain groups with specific characteristics regarding the land surface, number of livestock, irrigation, and frequency of herbicide use. Based on the responses, three management strategy subgroups were defined according to production systems: (1) rain-fed pastures, (2) irrigation with low stocking rate (<1 head/hectare), and (3) irrigation with high stocking rate (>1 head/hectare). The final classification consisted of nine ranches with rain-fed production, five ranches with low stocking rate, and six ranches with high stocking rate. At all ranches, groundwater depth ranged from 7 to 20 m (depth to shallow aquifer); all the selected ranches with irrigation have sprinkler irrigation systems with undefined schedules; some ranches irrigate in the early morning, while others irrigate at midday.

2.3. Sampling and Analysis

Samples were collected at different times since application, in April 2023 and September 2023, representing contrasting conditions in terms of seasonality (dry and rainy season, respectively). Regardless of the stocking rate subgroup, in September 2023, water was collected ten days after herbicide application, whereas in April 2023, herbicide application occurred six months prior to sample collection. Additional evidence of herbicide use came from empty herbicide containers observed in some locations (Figure 2). Water and soil samples were collected from all ranches. Water pipes were flushed two minutes before sampling. Water samples for 2,4-D quantification were collected directly from taps and well pumping systems that supply irrigation systems, using 500 mL amber glass bottles, washed with phosphate-free soap, acid-bathed (10% *v/v* HCl) for 24 h, then washed with deionized water, and rinsed with acetone. One additional sample was collected from a water well with no agricultural influence (21.161155° N, −87.995631° W) to distinguish

background contamination from agricultural sources. All samples were acidified with 2 mL of concentrated phosphoric acid for preservation purposes.



Figure 2. Containers of herbicides used in the area. From left to right: Tordon XT[®] (Aminopyralid + 2,4-D), Sendero[®] (2,4-D + Aminopyralid + Fluroxypyr-meptyl), Pastar[®] D (Aminopyralid + 2,4-D), and Velfosato[®] (Glyphosate).

The detection of 2,4-D was performed by High-Performance Liquid Chromatography with a diode array detector, using an Agilent 1290 Infinity HPLC-DAD instrument (Santa Clara, CA, USA) and Agilent OpenLAB CDS software (EZChrome Edition) version A04.05, Build 4.1.51.0. To obtain the 2,4-D identity peak, a calibration curve was obtained with 2,4-D PESTANAL[®] standard (analytical standard, Cat.# 31518, SUPELCO[®], Darmstadt, Germany) using concentrations of 250, 100, 50, 10, 5, 1, and 0.5 mg/L. As quality control, a second calibration curve was prepared with the same concentrations but with the commercial herbicide Pastar[™] D (DOW AGROSCIENCE de México; CORTEVA Agriscience, Jalisco, México) to identify the 2,4-D peak in commercial formulations. In addition, enriched samples were prepared with a concentration of 10 mg/L for both the analytical standard and commercial formulation. The results obtained with the standard and the commercial formulation served as a reference for identifying the retention time, the identity of the peak (Supplementary Figure S1), and finally determining the 2,4-D concentration in the samples.

The HPLC analytical conditions were adjusted following a published method [24]. The adjustments were 100% Acetonitrile as the mobile phase. The stationary phase used was a Discovery[®] RP Amide C16 column (5 µm, 180 Å, 15 × 2.1 cm, SUPELCO[®]). The flow rate was isocratic at 0.5 mL/min with an injection volume of 20 µL, and the temperature was controlled at 30 °C. Based on the literature, eight different wavelengths (210, 220, 230, 250, 260, 280, 310, and 350 nm) were monitored, with a total run time of 5 min for each injection. For the final quantification of herbicide, chromatograms at 220 nm were selected because the identity peaks showed better definition compared to the other wavelengths.

For the herbicide quantification from each groundwater sample, a solid-phase extraction (SPE) process was carried out using Strata[®] C18-E cartridges (55 µm, 70 Å). Cartridges were conditioned with 3 mL of methanol, washed with 1 mL of deionized water, loaded with 300 mL of sample, and finally eluted with 1.3 mL of acetonitrile in a 1.5 mL amber

glass vial. The limit of detection of the instrument (LOD) was 0.8 mg 2,4-D/L, and the SPE extraction recovery rate ranged from 66% to 114%.

Soil samples were collected from pasture plots with Guinea grass (*Panicum maximum*), *Brachiaria brizantha*, Taiwan grass (*Pennisetum purpureum* Schumach.) and Mombasa grass (*Panicum maximum* cv. Mombasa). PVC tubes 20 cm long and 5 cm in diameter were used for soil collection (Figure 3). The samples represent the top 20 cm of soil.



Figure 3. Soil sampling at the study sites in the agro-industrial karst zone of northeastern Yucatán.

Organic matter was obtained by loss-on-ignition and [25] and soil organic carbon was estimated by adjusting the above-mentioned results using the equation $OM = \% C.org \times 1.724$. The amount of organic matter obtained using this method represents between 76 and 80%; therefore, the Van Bemmelen correction factor (1.724) is used for greater accuracy in the results [26]. Cation exchange capacity is considered an indirect indicator of soil buffering capacity [27]. This parameter was determined using the titration method based on ion exchange at a buffered pH, where the ammonium ion (NH_4^+) acts as the index saturating cation; the procedure involves four sequential steps: saturation of exchange sites (with an ammonium acetate buffered solution), removal of excess reagent, displacement of adsorbed NH_4^+ and a final quantification with volumetric titration [28]. The values obtained were categorized according to the Mexican Standard NOM-021-SEMARNAT-2000 [6], as shown in Table 1.

Table 1. Organic matter (OM) values in mineral soils and cation exchange capacity (CEC in $cmol_c/kg$) as indicated by NOM-021-SEMARNAT-2000.

Level	OM Mineral Soils (%)	CEC ($cmol_c/kg$)
Very high	>6	>40
High	3.6–6	25–40
Medium	1.6–3.5	15–25
Low	0.6–1.5	5–15
Very low	<0.5	<5

2.4. Statistical Analysis

Statistical analyses and graphs were performed using Origin Pro 2021 and SPSS 13 software. The three variables evaluated (organic matter, cation exchange capacity, and 2,4-D concentration) were subjected to the Shapiro–Wilk normality test, which indicated that normality was rejected for the three variables and were analyzed by the non-parametric Kruskal–Wallis method and the Dunn test ($p > 0.05$).

3. Results and Discussion

3.1. 2,4-D Concentration

Significant differences were detected between sampling events (time since application ($\chi^2 = 18.97$, $p < 0.00001$), likely because applications for weed control were carried out after the first rain events following the end of the dry season (June–July), which, combined with irrigation, caused leaching of agrochemical residues from the soil into the shallow aquifer. Furthermore, lower herbicide application and the absence of rainfall during the dry season (April 2023) resulted in less herbicide leaching into the aquifer, coupled with lower evapotranspiration [29]. Regarding management strategies, rainfed ranches showed greater variability, and differences were observed when compared to ranches with irrigation, both at low and high stocking rates ($\chi^2 = 37$, $p < 0.0001$, Figure 4). Ranch managers reported that agrochemicals were applied throughout much of the year to create firebreaks and to prevent forest fires, which may result in residual herbicides from previous seasons, even at low concentrations.

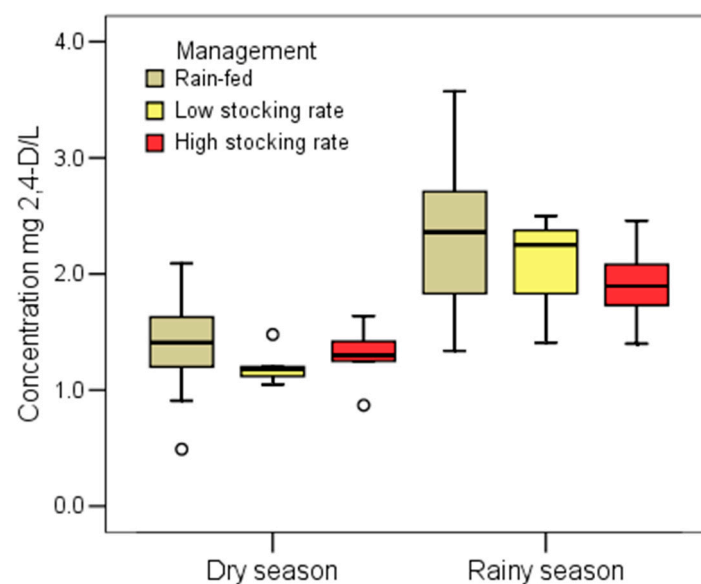


Figure 4. Herbicide concentration in mg 2,4-D/L by ranch management strategy category in the agro-industrial karst zone of northeastern Yucatán. Low stocking rate <1/ha: head of cattle per hectare). Dry season represented by sampling in April 2023 and rainy season represented by sampling in September 2023.

2,4-D is one of the most used herbicides for weed control in grasses in the state of Yucatán [5,30]. Its presence in groundwater has already been reported, where most samples analyzed in 2018 were below 0.5 mg 2,4-D/L [31]. The concentrations quantified in the agricultural zone in September 2023 reached a maximum of 3.6 mg/L, while levels during the April 2023 sampling event were lower, but still very high (2.1 mg/L), given the recommended values in other countries [32,33]. Higher 2,4-D concentrations in groundwater could have been the result of a combination of greater application intensity, changes in rainfall patterns, and most of all, differences in management and stocking rates (our sampling sites were not the same as those reported by [31]).

The concentration of the herbicide 2,4-D was different between the sampling events, likely the result of differences in time since application, together with downward movement (infiltration) with rainwater and irrigation. Irrigation systems increase the infiltration of contaminants into the soil, and ultimately into the aquifer [34,35]. Irrigation combined with the high vulnerability of the aquifer [36] plays a significant role in the infiltration of agricultural residues into groundwater, facilitating the entry of various contaminant

substances [37]. Establishing a groundwater monitoring strategy would enable early detection of contaminants, application guides, the creation of buffer zones and application-rate decisions, to protect water sources and support sustainable intensification of cattle production without compromising environmental integrity [12].

3.2. Soil Characterization

Organic matter content did not differ by management strategy ($\chi^2 = 0.401$, $p = 0.81$); therefore, the hypothesis that ranch management (number of cattle per hectare) influences the amount of available soil organic matter (OM) was rejected (Figure 5). However, there are differences between seasons ($\chi^2 = 28.29$, $p > 0.0001$), suggesting that the soil's attenuation capacity with respect to agricultural residues may vary with the season.

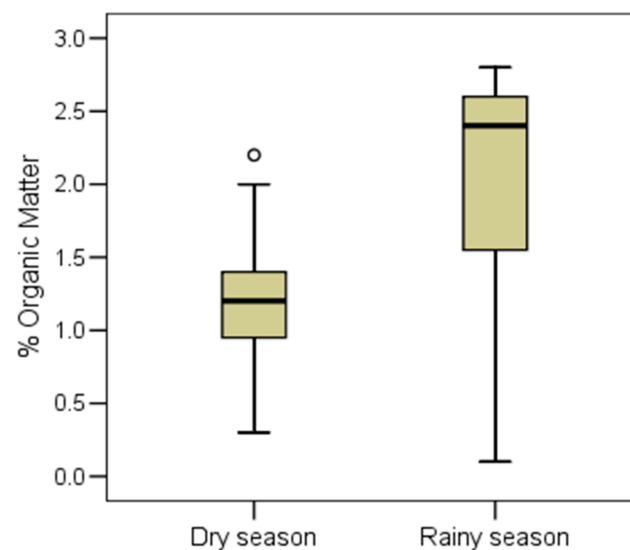


Figure 5. Box and whisker plot of organic matter content (%) in the agro-industrial karst zone of northeastern Yucatán. Dry season represented by sampling in April 2023 and rainy season represented by sampling in September 2023.

Greater amounts of organic matter could have influenced the retention of contaminants, preventing them from reaching the aquifer [38]. Nonetheless, even with higher SOM, leaching could intensify due to rain [39], carrying agricultural residues into the aquifer. It should be noted that 2,4-D is highly soluble in water, has relatively low absorption in soil, and moderate mobility; therefore, its presence is assumed to be directly related to the high frequency of applications.

The effectiveness of herbicides depends largely on their chemical and physical properties and can be reduced by adsorption into the soil. Acidic herbicides such as 2,4-D are partially ionized and can bind to soil colloids; changes in soil pH could make them more available in solution [8]. Once in solution, it has high mobility potential, with a high Ground Ubiquity Score (GUS) of 3.906 [40], and an organic carbon partition coefficient (Koc) of 61 mL/g [9], considered low, thus with a high potential to be mobile in soil, likely leaching into groundwater. The degree and intensity of adsorption in soil are determined by the texture, quantity, and type of clay and organic matter, as well as the moisture content; however, organic matter seems to be the most important factor [9,41] since carbonates have not been reported to retain [42] or have any positive effect on the degradation of this herbicide [43]. Porosity, exchangeable sodium percentage, calcium-to-magnesium ratio, and parent material are also factors that determine soil stability [44].

Overgrazing of pastures is one of the main causes of degradation and loss of structure in agricultural soils, reducing vegetation cover, causing an increase in temperature,

raising evapotranspiration levels, and reducing the available moisture for plants [45,46]. Additionally, irrigation and rainwater can also contribute to the loss of soil structure due to the “raindrop impact” effect, causing its disintegration [47]. Therefore, if the soil is devoid of vegetation cover or organic matter, there might be losses of nutrients and clay, as well as leaching of other substances [48,49].

Soil organic matter is related to soil water-holding capacity (WHC) to a given extent [50]. In prolonged meteorological drought, agronomic drought follows, exacerbated by the absence of vegetation cover [51]. Drought reduces the production of aboveground dry matter and its decomposition for incorporation into the soil by up to 30%, thus decreasing the amount of soil organic matter [52].

The cation exchange capacity (CEC, Figure 6) had no significant differences found between seasons ($\chi^2 = 1.31$, $p = 0.252$). During the dry season, the minimum and maximum CEC values were 12.9 cmol_c/kg and 23.3 cmol_c/kg, respectively; while during the rainy season, the minimum was 14.6 cmol_c/kg, and the maximum was 22.3 cmol_c/kg. Conversely, statistically significant differences were found in CEC values evaluated by stocking rate ($\chi^2 = 10.29$, $p = 0.0058$), suggesting changes in soil nutrient availability. One of the distinctive characteristics of CEC is its dependence on clays and organic matter, and this parameter changes as the soil organic matter and pH change, which in turn depend on the irrigation depth [53,54]. It should be considered that increasing or decreasing the amount of organic matter also changes the availability of essential elements for crop development [55].

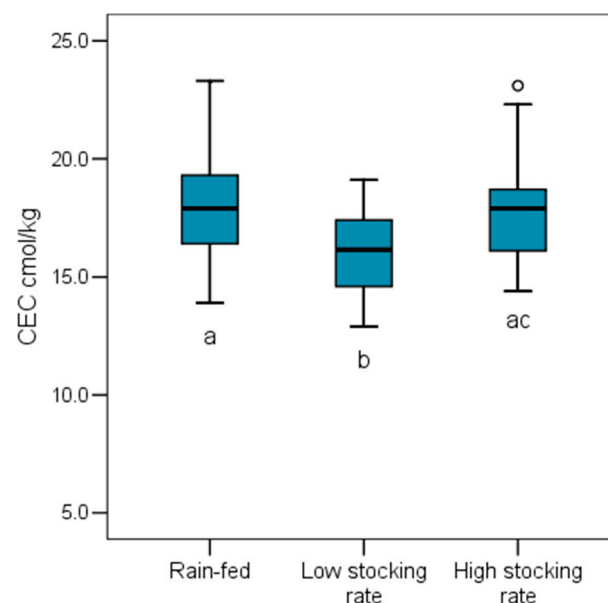


Figure 6. Cation exchange capacity (CEC) by ranch management strategy category in the agro-industrial karst zone of northeastern Yucatán. Ca/ha: head of cattle per hectare. Lower case letters in the figure represent statistically significant differences found in CEC by stocking rate.

4. Conclusions

With the data we obtained, we cannot conclude that irrigation water quality is influenced by stocking rate. The concentration of 2,4-D was higher during September 2023; yet the presence of herbicide during April 2023 may be due to leached herbicide from past applications or infiltration of residues due to irrigation. The soils have low to medium organic matter content, which, together with the average cation exchange capacity (CEC) values, suggests low retention of 2,4-D in the soil. During the rainy season, higher herbicide concentrations were measured due to active application, rain and irrigation, causing vertical movement into the aquifer, even though the soils had higher amounts of organic

matter and greater CEC. Irrigated agriculture is one of the main activities that alter water quality worldwide. It is recommended to reduce overgrazing in pastures to decrease soil compaction and prevent losses of organic matter and cation exchange capacity, thus avoiding negative effects on aquifers. Monitoring 2,4-D concentrations in groundwater is essential because this herbicide is not easily biodegradable and is frequently detected in groundwater, particularly near agricultural areas. Training and integrating food security with environmental security will help to jointly preserve the quality of human life, the quality of food produced, and the conservation of vital resources such as soil and water.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture16151584/s1>, Figure S1: spiked sample; Table S1: GPS Coordinates.

Author Contributions: Conceptualization, M.G.-M. and E.C.; methodology, M.G.-M. and J.E.B.-G.; investigation and formal analysis, M.G.-M. and E.C.; resources, R.M.L.-B. and E.C.; writing—original draft preparation, review and editing, M.G.-M., J.E.B.-G., R.M.L.-B. and E.C. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original data presented in the study are openly available in Repositorio CICY at <https://cicy.repositorioinstitucional.mx/jspui/handle/1003/3025> (accessed on 10 July 2026).

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Conflicts of Interest: The authors declare no conflicts of interest.

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