



Eco-toxicological and Nutritional Hazards of Tembotrione: A Multi-Species Evaluation After Long-Term Use

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Abstract— Environmental contamination from herbicide applications for weed control remains a significant concern. This study presents an ecological and dietary risk assessment of tembotrione following its long-term use in maize cultivation, focusing on potential impacts on humans, animals, and aquatic organisms. Residue levels and degradation behavior of tembotrione were monitored in soil, maize plants, and grains at various intervals post-application to estimate exposure risks. The half-life showed a positive correlation with tembotrione persistence in soil, as well as with its water solubility and volatility. Risk Quotient (RQ) values indicated a risk ranging from high to negligible for soil macro-organisms over a 60-day period, with a moderate risk at 90 days. Health Quotient analysis revealed that the risk to animal health from consuming contaminated maize straw varied from high to low. Human dietary exposure posed a relatively low risk. However, aquatic organisms exhibited moderate to high ecological risk. These findings underscore the importance of tembotrione residues and its long-term ecological footprint in agricultural systems.

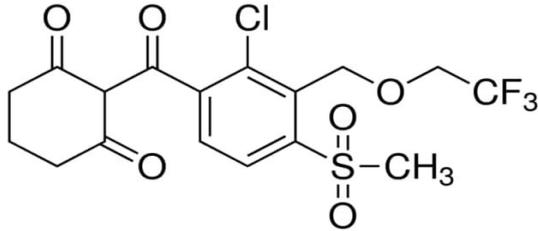
Keywords— Risk assessment; Fish; Health; Macro-organisms; Pesticides; Maize cultivation; Herbicide persistence.

I. INTRODUCTION

Herbicides are among the most widely used pesticides for effective weed control, with global consumption estimated at approximately 1.4 million metric tons annually (Statista, 2020). However, their frequent application has led to growing concerns, including the development of herbicide-resistant weed species, phytotoxic effects on non-target crops, and the accumulation of chemical residues in soil and aquatic ecosystems (Zhang et al., 2019; Wang et al., 2022; Heap, 2023; Sahand et al., 2021; Sondhia & Waseem, 2020; Syafrudin et al., 2021). As a result, many herbicides have been banned or subjected to regulatory restrictions in various countries (Sondhia, 2014; 2018; Zhou et al., 2019; Sondhia & Waseem, 2020; Syafrudin et al., 2021). Maize, the second most extensively cultivated crop worldwide, occupies around 193.7 million hectares with an average yield of 5.75 tons per hectare (FAOStat, 2020; ICAR-IIMR, 2022). Looking ahead, maize is projected to dominate global cereal consumption for animal feed, growing at an annual rate of 1.3%. By 2031, global maize production is expected to increase by 161 million tons, reaching approximately 1.33 billion tons (OECD-FAO, 2022).

Tembotrione is a triketone-class herbicide widely used for post-emergence weed control in maize cultivation across the globe (Christos et al., 2018; Khanna et al., 2022). Its mode of action involves inhibition of the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD), which disrupts carotenoid biosynthesis in target weed species. The global market value of tembotrione was estimated at USD 33.4 million in 2020 and is projected to grow at a compound annual growth rate (CAGR) of 13.6%, reaching USD 81.54 million in the coming years (Global Tembotrione Market, 2023). Chemically, tembotrione is hydrolytically stable and non-volatile (EPA, 2007; Zemelka, 2015), with a water solubility of 28.3 g/L at pH 7 (PubChem NCBI, 2023; Table 1). It undergoes transformation into xanthenedione derivatives, which exhibit greater toxicity than the parent compound (Wang et al., 2022).

TABLE 1
IMPORTANT PHYSICO-CHEMICAL PROPERTIES OF TEMBOTRIONE

| | |
|---|--|
| Tembotrione chemical structure |  |
| Chemical name | Tembotrione, 2-[2-chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]benzoyl]-1,3-cyclohexanedione |
| molecular formula | C17H16ClF3O6S |
| Mol wt g/mol | 440,82 |
| Melting point | 117 °C |
| pH @ 24 °C | 3.63 |
| Water solubility (g/L @ 20 °C) | 28.3 at pH 7 |
| Vapor pressure (Torr, 20 °C) | 8.25 x 10 ⁻¹¹ |
| Dissociation constant (pKa) | 3.2 (means weak acid) |
| Octanol/water partition coefficient (Pow @ 24 °C) | 0.0807 at pH 7.0 |
| Acute Oral – rat LD50 (mg/kg) | > 2000 |
| Tolerance Levels: | |
| Corn, field, grain | 0.03 ppm |
| Corn, field, forage | 0.60 ppm |

Concerns have emerged regarding tembotrione's residual persistence in soil, with some studies reporting its presence for up to 200–300 days post-application (PMRA, 2012). Such persistence has been linked to phytotoxic effects on sensitive rotational crops including potato (Dias et al., 2019), beet (Silva et al., 2019), and carrot (Bontempo et al., 2016). Additionally, tembotrione has been shown to negatively impact soil biological health by reducing dehydrogenase and alkaline phosphatase enzyme activities, suppressing arbuscular mycorrhizal fungi populations, and impairing root colonization (Goalla et al., 2022).

Although chemical weed control methods offer efficient alternatives to manual weeding and enhance overall weed management, they also raise concerns regarding environmental safety. In particular, the long-term persistence and residue behavior of herbicides like tembotrione in soil, plant tissues, and water bodies warrant comprehensive investigation. Existing studies have primarily focused on short-term degradation patterns and residue mobility, often overlooking the extended dietary risks associated with consuming maize cultivated in tembotrione-treated fields. These earlier assessments have emphasized residue concentrations and leaching potential, without evaluating chronic exposure effects on humans and non-target organisms.

To address this gap, the present study aims to evaluate the ecological and long-term dietary risks of tembotrione through a multi-year analysis of its residues in maize soil, plant tissues, water, and aquatic organisms. Conducted over three consecutive growing seasons, this research provides a more holistic understanding of tembotrione's environmental footprint and its implications for food safety and ecosystem health.

II. MATERIALS AND METHODS

2.1 Field experiment:

Field experiments were conducted during the rainy seasons of 2017, 2018, and 2019 on black cotton soils (Vertisols) to evaluate the effects of tembotrione application in maize cultivation. The soil at the experimental site was classified as sandy clay loam, comprising 66.5% sand, 23.3% clay, and 10.2% silt, with an electrical conductivity (EC) of 200 µS/m, pH of 7.01, and organic carbon content of 0.89%. A hybrid maize variety (4212) was cultivated following standard agronomic practices recommended

for the central province. Tembotrione was applied as a post-emergence herbicide at the recommended dose of 120 g active ingredient per hectare (g a.i./ha) during the maize growing seasons. Control plots were maintained without herbicide application. Each experimental plot measured 16 meters in length and 10 meters in width, with raised soil boundaries approximately 30 cm in height and width on all sides to prevent cross-contamination. Meteorological data for the study years (2017–2019) is presented in Figure 1.

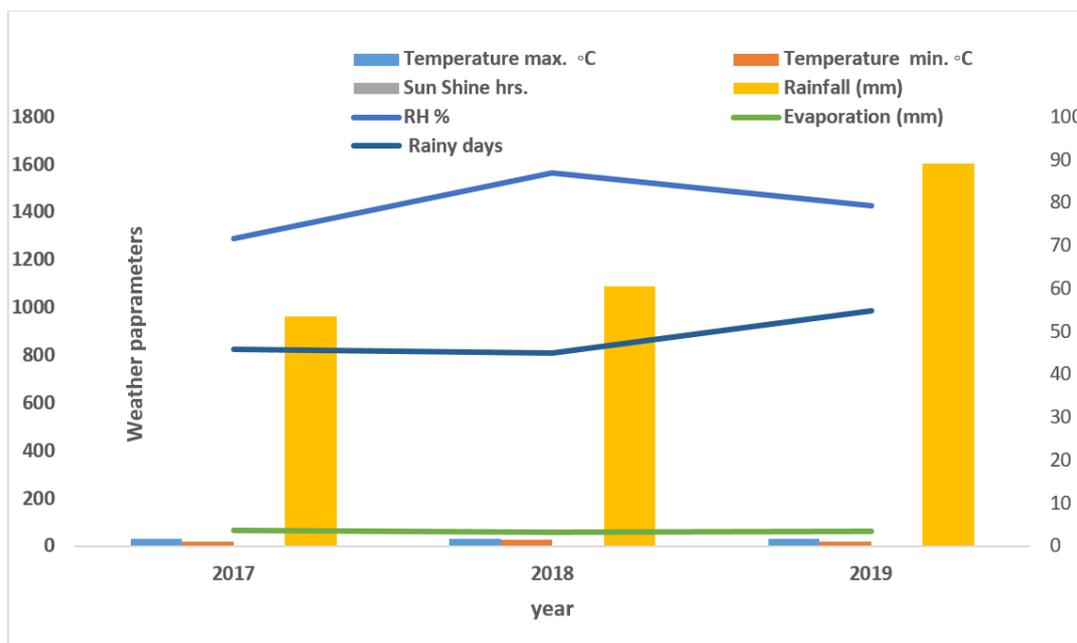


FIGURE 1: Minimum and maximum temperature, relative humidity, sunshine, and total rain during the maize growing stage period in each year

Periodic sampling of soil (0–15 cm depth), maize plants, and water was conducted from 2 hours after the application of tembotrione up to harvest. Mature maize plant samples were collected at harvest from both treated and untreated plots to compare residue levels. To assess bioaccumulation and estimate tembotrione residues in aquatic organisms, fish samples were collected from neighboring ponds between 20 and 100 days after herbicide application. Fish mortality was observed in adjacent ponds receiving tembotrione through drift and surface runoff from treated maize fields. All collected samples were appropriately stored and processed for tembotrione residue analysis using Ultra-Fast Liquid Chromatography (UFLC).

2.2 Herbicide extraction and quantification:

Tembotrione was procured in two forms—analytical grade standard and commercial formulation (Tembotrione 34.4% SC)—from Bayer Crop Sciences India Pvt. Ltd. All analytical-grade chemicals and solvents used in the study were obtained from Merck (Darmstadt, Germany). Quantification of tembotrione residues in soil, water, fish, and maize plant samples was performed using a validated Ultra-Fast Liquid Chromatography (UFLC) method, as described by Sondhia (2016). The analytical procedure was validated in accordance with WHO (2020) and SANTE (2021) guidelines, assessing key parameters including recovery rates at 0.1 mg/L and 0.5 mg/L, selectivity, linearity, limits of detection (LOD) and quantification (LOQ), precision, and accuracy.

Tembotrione residues in soil, water, fish, and maize samples were analyzed using a Prominence UFLCXR system (Shimadzu, Japan), equipped with an SPD-MP00A VP diode array detector, LC-c0 AD pump, DHU degasser, 20A3 unit, and an autosampler. Chromatographic separation was achieved on an XR ODS II column (75 mm length × 3 mm internal diameter) using a mobile phase composed of acetonitrile and water containing 0.01% H₃PO₄ in a 70:30 (v/v) ratio. The flow rate was maintained at 0.35 mL/min. A 20 µL aliquot of tembotrione standard solution (ranging from 0.001 to 10 mg/L in acetonitrile) was injected via the autosampler. Detection was performed at 231 nm, and peak areas (µabs) were recorded.

Under these conditions, tembotrione exhibited a retention time of 3.13 minutes, with a total run time of 6.5 minutes per sample. Calibration was performed by plotting peak area (µabs/sec) against tembotrione concentration (µg/mL), and the data were fitted to a linear regression model to generate a standard curve. Tembotrione concentrations in test samples were calculated using the regression equation: $y = 30788x + 43543$ with a correlation coefficient $R^2 = 0.998$, indicating excellent linearity.

2.3 Risk assessment:

Tembotrione residues detected over time in soil, water, maize plants, and fish were analyzed to assess the ecological risk and potential toxicity to various taxa. This evaluation followed the frameworks established by EFSA (2019), FAO/WHO, and the EPA Risk Assessment Guide (2022), utilizing toxicological reference data from EPA (2007), EFSA (2013), and USDA (2022). The ecological risk was quantified using the Risk Quotient (RQ) method, wherein calculated RQ values were compared against the EPA's Level of Concern (LOC). According to EPA guidelines, the risk is considered acceptable when $RQ < LOC$. The RQ approach, widely adopted for preliminary environmental screening, serves as a standardized tool to estimate the potential hazard of pesticide residues across environmental compartments.

A risk quotient (RQ) as prescribed by the US Environmental Protection Agency is used to evaluate the ecological risk of pesticides (Equation 1).

$$\text{Risk Quotient} = \text{Exposure} / \text{Toxicity} \quad (1)$$

In the referenced equation, exposure refers to the Estimated Environmental Concentration (EEC), while toxicity corresponds to an effect level or endpoint derived from ecotoxicological studies, such as the median lethal concentration (LC_{50}) or the No Observed Effect Concentration (NOEC). Level of Concern (LOC) values were calculated using EEC in conjunction with LC_{50} or NOEC data, following the methodology prescribed by the U.S. Environmental Protection Agency (EPA) and adopted by Shobha et al. for ecological risk assessment. Additionally, dietary exposure to tembotrione was evaluated in accordance with World Health Organization (WHO) guidelines and Dietary Risk Index (DRI) was calculated as described by Benbrook and Davis (2020) and given as equation 2:

Dietary exposure = Σ (Concentration of pesticide in food \times Food consumption) / Body weight (kg)

$$\text{DRI} = (\text{Pesticide concentration} \times \text{Serv}) / (\text{cRfd} \times \text{BW}) \quad (2)$$

Where, Pesticide concentration (mg/kg); Serving (kg), cRfd (mg/kg bw); BW Body weight (kg).

And the risk characterization for tembotrione was performed by estimation of the Hazard Quotient (HQ), i.e., the estimated total chronic dietary exposure divided by the Acceptable Daily Intake (ADI) (mg/kg bw). Ecological and health assessment was described based on correlation among the calculated dietary risk exposure assessment and residues of tembotrione detected in various samples taken in this study where tembotrione was applied continuously for three years at recommended dose of 120 g/ha.

2.4 Data analysis:

Regression analyses were performed using Microsoft Excel's graphing tools and data analysis functions on the Windows platform. The dissipation behavior of tembotrione in maize field soil was characterized through a linear regression model. Residue concentrations were expressed as mean \pm standard deviation (S.D.) and statistically evaluated using analysis of variance (ANOVA). Significant correlations between mean tembotrione residues, soil physico-chemical properties, and meteorological parameters were assessed using a Pearson correlation matrix. The dissipation kinetics of tembotrione were modeled using a first-order reaction equation: $C_t = C_0 e^{-kt}$, where C_t (mg/kg) denotes the concentration at time t (min), C_0 (mg/kg) the starting concentration in the soil, and k (per minute) is the rate constant.

III. RESULTS AND DISCUSSION

3.1 Dissipation and degradation behavior of tembotrione in maize field soil:

Ultra-Fast Liquid Chromatography coupled with a diode array detector (UFLC-DAD) was employed for the quantification of tembotrione residues across various environmental and biological matrices, owing to its high sensitivity and suitability for multi-residue pesticide analysis. The method demonstrated satisfactory recovery rates ranging from 81% to 92% across maize plants, grain, straw, soil, water, and fish samples. The relative standard deviation (RSD) for all matrices was below 5%, indicating good analytical precision. The limit of detection (LOD) for tembotrione was determined to be 0.001 mg/kg.

In soil, tembotrione dissipation was monitored over three consecutive years following its application in maize fields. In 2017, an initial concentration of 0.7176 mg/kg was detected two hours post-application, which declined to 0.4425 mg/kg at five days, 0.2239 mg/kg at 20 days, and 0.077 mg/kg by 90 days (Figure 2). In 2018, residues measured 0.641 mg/kg at two hours, decreasing to 0.443 mg/kg at 10 days, 0.1925 mg/kg at 20 days, and 0.010 mg/kg by 90 days. Similarly, in 2019, the initial

residue concentration was 0.541 mg/kg, which dissipated to 0.0223 mg/kg at 60 days and 0.010 mg/kg at 90 days. By harvest in all three years, residue levels in soil were consistently below the LOD (<0.001 mg/kg).

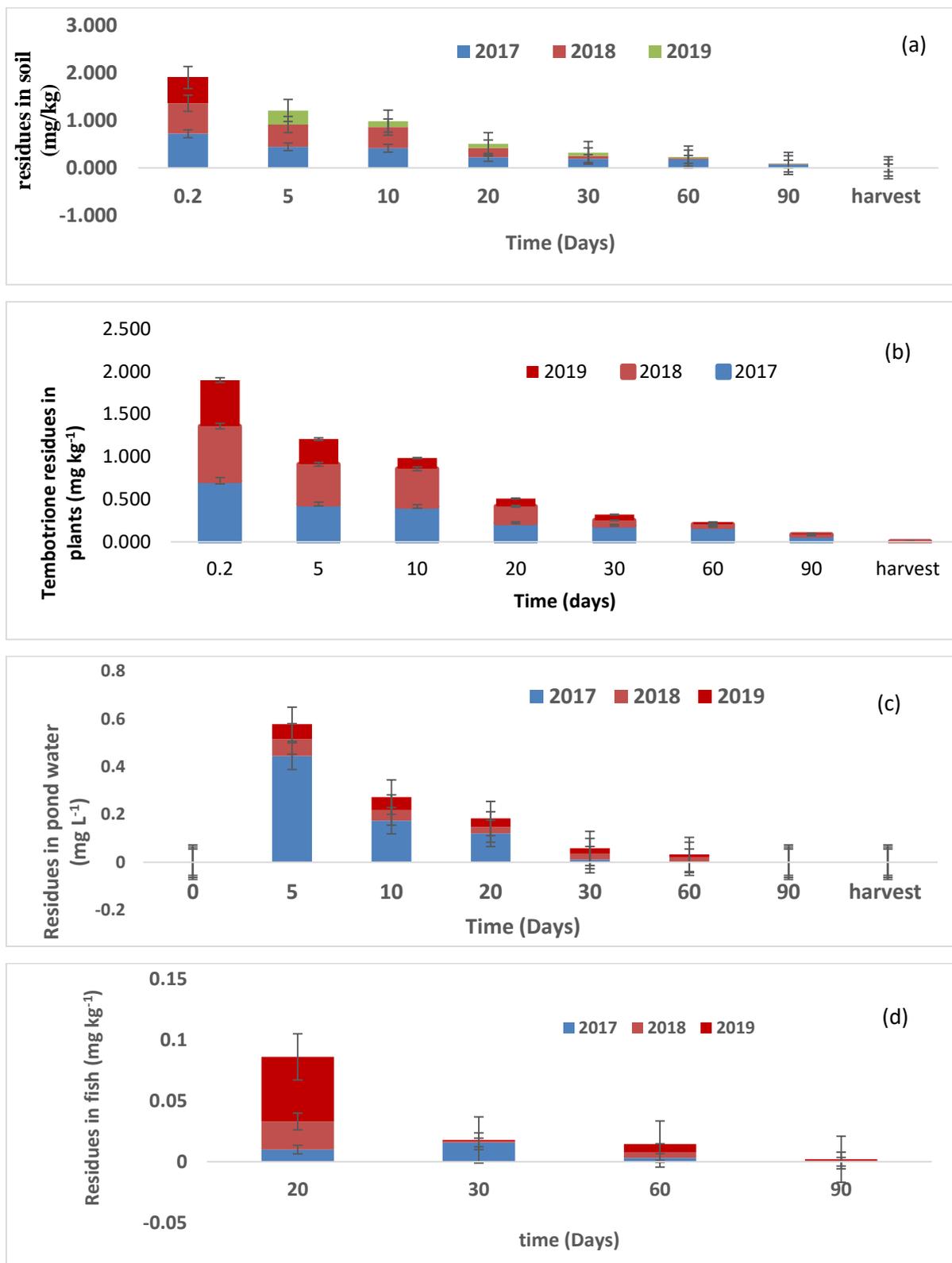


FIGURE 2: Detection of tembotrione residues in the soil of maize field (a), maize plants (b) adjacent pond water (c) and fishes (d) during 2017, 2018 and 2019

The dissipation of tembotrione in soil followed first-order kinetics, with regression equations for each year as follows:

- 2017: $Y = -0.0442x + 4.3009$ ($R^2 = 0.710$)
- 2018: $Y = -0.0560x + 4.1095$ ($R^2 = 0.941$)
- 2019: $Y = -0.0496x + 3.5251$ ($R^2 = 0.919$)

Corresponding half-lives of tembotrione in soil were calculated as 15.67, 12.38, and 13.97 days for 2017, 2018, and 2019, respectively (Table 2; Figure 3). The average half-life across the three years was estimated at 14.0 days. By 90 days post-application, more than 90% of the applied tembotrione had degraded in all study years (Figure 4), indicating its relatively rapid dissipation under field conditions.

TABLE 2
RATE KINETICS EQUATIONS AND HALF-LIFE OF TEMBOTRIONE AND IN THE SOIL OF MAIZE FIELD AND MAIZE PLANTS DURING 2017 TO 2019

| Herbicides | Rate kinetics equation | R ² | Calculated time for 50% Dissipation | Average Half-life (days) |
|---------------------|-------------------------|----------------|-------------------------------------|--------------------------|
| Soil | | | | |
| 2017 | $Y = -0.0442x + 4.3009$ | 0.71 | 15.67 | 14 |
| 2018 | $Y = -0.056x + 4.1095$ | 0.941 | 12.37 | |
| 2019 | $Y = -0.0496x + 3.5251$ | 0.919 | 13.97 | |
| Maize plants | | | | |
| 2017 | $Y = -0.0749x + 5.2194$ | 0.982 | 9.252 | 11.4 |
| 2018 | $Y = -0.0553x + 4.9684$ | 0.935 | 12.53 | |
| 2019 | $Y = -0.0551x + 4.2900$ | 0.801 | 12.57 | |

3.2 Tembotrione degradation in maize plants, grain and straw:

Tembotrione residue levels in maize plants were monitored over three consecutive years following application for weed management. Initial residues detected at one-day post-application were 0.718, 0.641, and 0.541 mg/kg in 2017, 2018, and 2019, respectively. These concentrations progressively declined to 0.180, 0.027, and 0.022 mg/kg by 60 days, indicating substantial dissipation. By 90 days, residue levels were further reduced to 0.077 mg/kg (2017) and 0.010 mg/kg (2018 and 2019).

The dissipation of tembotrione in maize plants followed first-order kinetic models, with calculated half-lives of 9.25, 12.53, and 12.57 days in 2017, 2018, and 2019, respectively (Table 2). Rapid metabolic degradation of tembotrione was observed in the maize plants, particularly during the early growth stages. More than 90% of the initial residues were degraded within 60 days in 2018 and 2019, while similar levels of degradation were reached by 90 days in 2017.

At harvest, tembotrione residues in both maize grain and straw were below the analytical method's limit of detection (0.001 mg/kg), indicating the absence of residual contamination in edible parts of the plant (Figures 3 and 4).

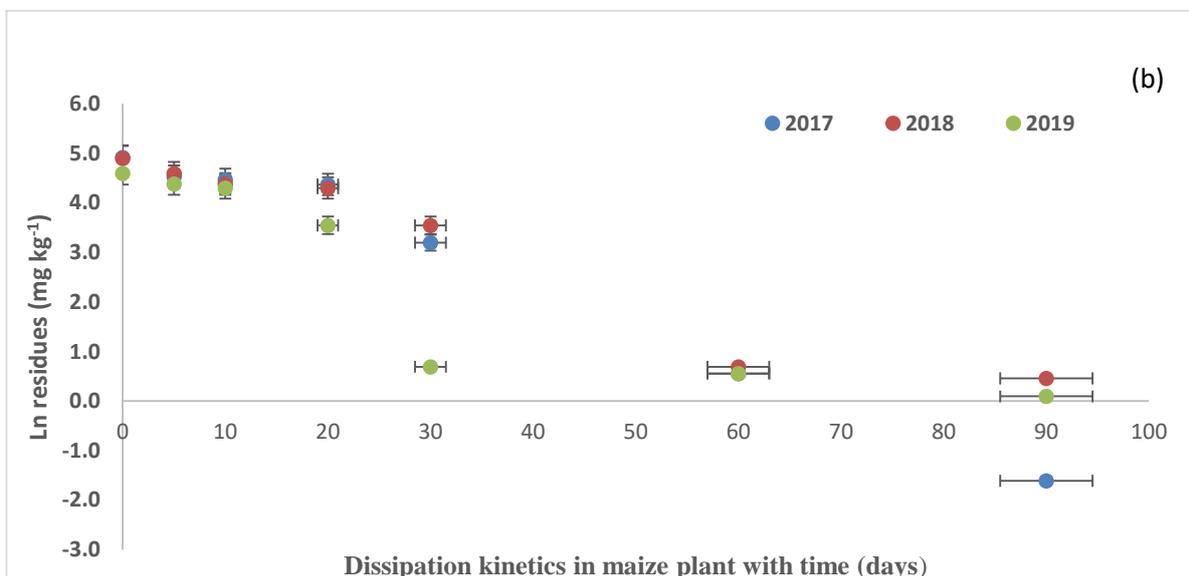
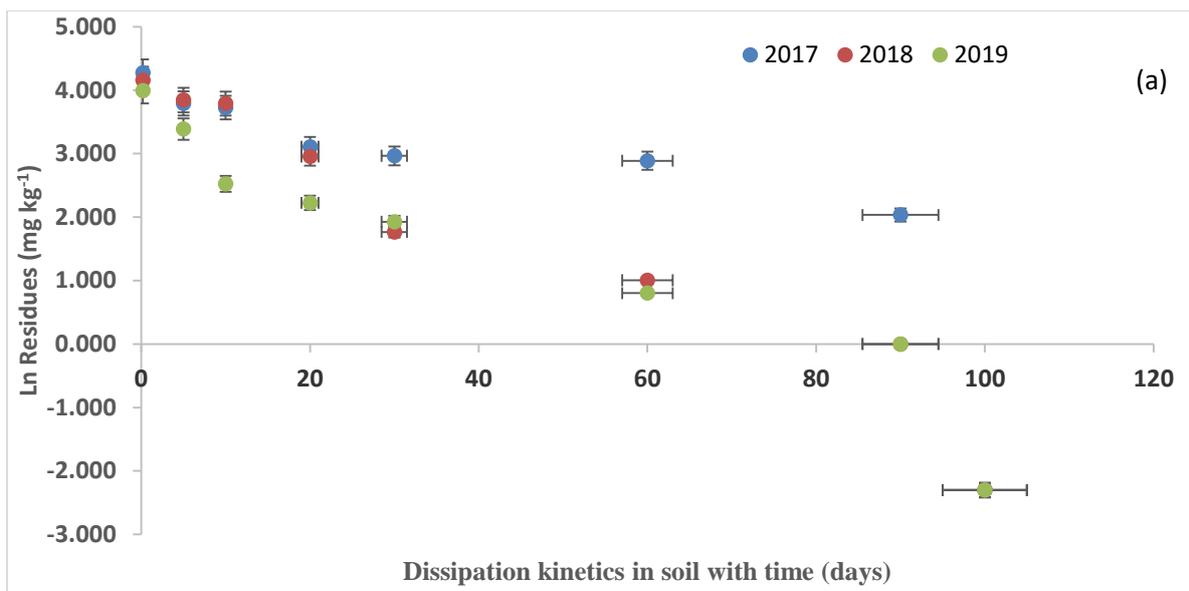
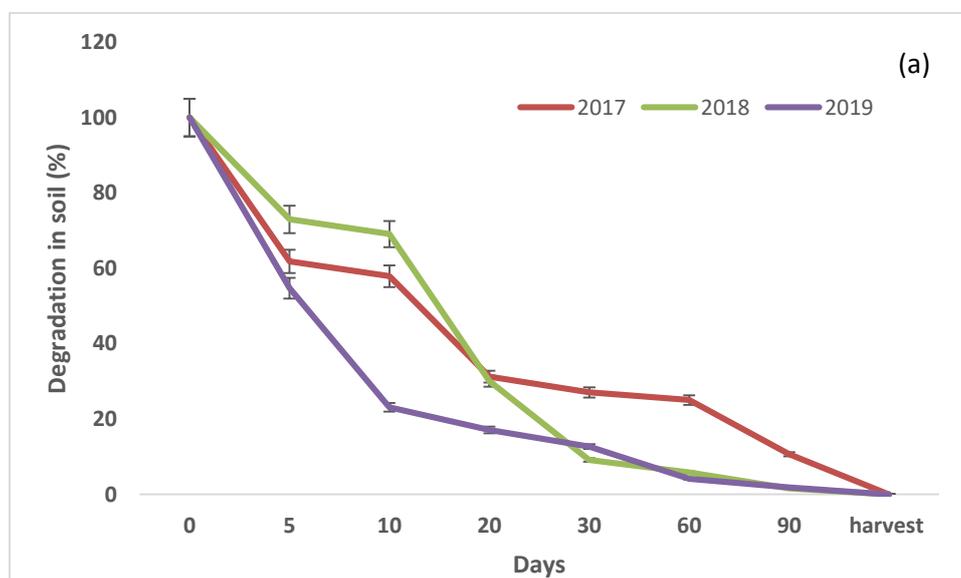


FIGURE 3: First order dissipation pattern of tembotrione residues in the soil of maize grown field (a) and maize plant (b) during 2017, 2018 and 2019



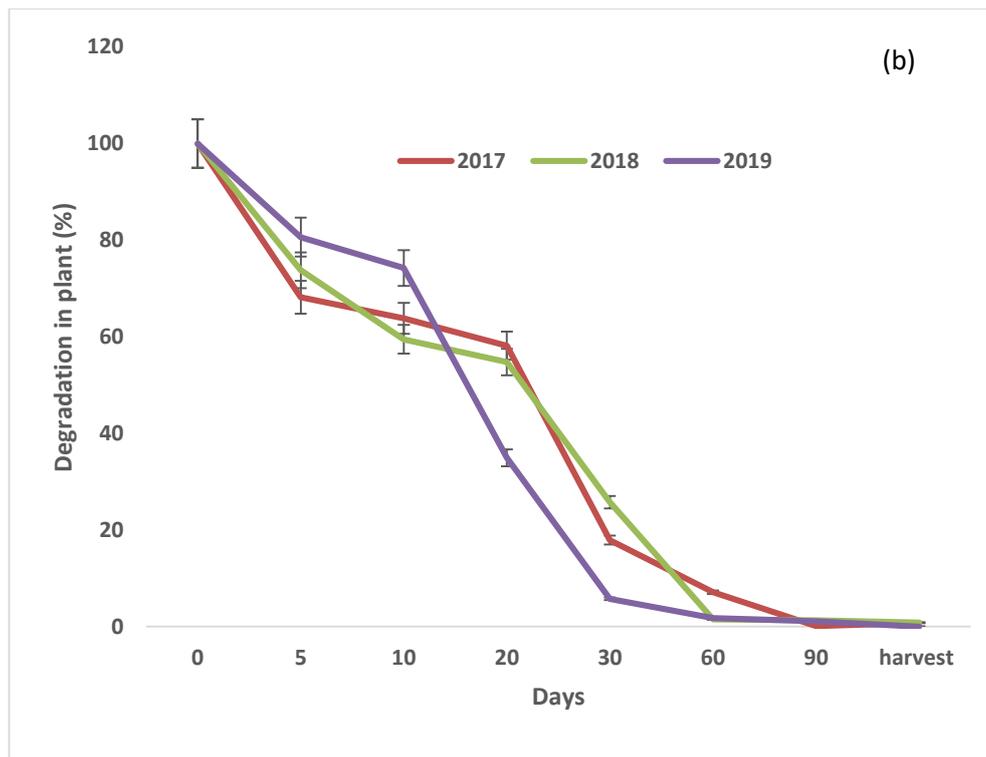


FIGURE 4: Tembotrione degradation residues in maize field soil (a) and maize plants (b) during the years 2017, 2018, and 2019

3.3 Tembotrione residues and mortality in the fish:

Tembotrione residues were detected in water samples from a nearby pond following application in maize fields. In the first year, residue levels reached 0.444 mg/L at 5 days post-application and declined to 0.0121 mg/L by day 20. In the second year, concentrations ranged from 0.0720 mg/L at 5 days to 0.0261 mg/L at 30 days. During the third year, residues varied between 0.0619 mg/L and 0.0361 mg/L at 5 and 20 days, respectively (Figure 2). Across all three years (2017–2019), residue levels in pond water dropped to below 0.001 mg/L by 90 days post-application.

Residue accumulation and potential toxicological effects on fish were also evaluated. Tembotrione was transported into the adjacent pond via rainwater runoff during the experimental period. Consequently, residue levels in fish muscle tissue at 60 days were recorded as 0.067, 0.0048, and 0.0031 mg/kg in 2017, 2018, and 2019, respectively. These levels suggest that fish may not be suitable for consumption at this stage. However, residue concentrations declined to below the detection limit (0.001 mg/kg) by 90 days in all years.

Importantly, no instances of fish mortality or observable toxic symptoms were recorded in the pond on any sampling day throughout the study period.

3.4 Risk assessment of tembotrione in aquatics and other taxon:

Risk quotient (RQ) values were calculated based on field-derived residue data, and the results are presented in Table 3. The acceptable daily intake (ADI) for tembotrione is 0.0004 mg/kg body weight (bw) (EPA, 2007; EFSA, 2013). For dietary risk assessment, an average body weight of 65 kg for humans and 450 kg for cattle was assumed, in accordance with EFSA (2019) and as adopted by Benbrook et al. (2020). The RQ values for all assessed organisms were found to be well below 1 and remained below the established levels of concern (LOC), indicating minimal ecological and human health risks.

TABLE 3

Risk assessment based on Risk quotient and level of concern of tembotrione on aquatic life and other taxon based on residues detected in the adjacent aquatic ponds near to maize field where tembotrione was applied at recommended dose continuously for three years (risk assessment was calculated based on residues as well as NOEC data available at Environmental Protection Agency (EPA) or European Food and Safety Assessment, (EFSA) website

| Organism | Selected species | NOEC (mg/L) | LOC Presumptions | RQ Presumptions | LOC | RQ _r Risk assessment based on mean residues (mg/L) in water at various days | | | | | | |
|----------------------------------|--|-------------|------------------|------------------------|----------------|--|--------|--------|--------|--------|--------|--------|
| | | | | | | 0.2 | 5 | 10 | 20 | 30 | 60 | 90 |
| Fathead minnow | <i>Pimephales promelas</i> | 0.604 | ARU | Moderate | >0.05 to <0.5 | NA | Yellow | Yellow | Yellow | Blue | Blue | Green |
| Rainbow trout | <i>Oncorhynchus mykiss</i> | 100 | NR | Negligible | <0.05 | NA | Green | Green | Green | Green | Green | Green |
| water flea | <i>Daphnia magna</i> | 18 | NR | Negligible | <0.05 | NA | Green | Green | Green | Green | Green | Green |
| Bluegill sunfish | <i>Lepomis macrochirus</i> | 100 | NR | Negligible | <0.05 | NA | Green | Green | Green | Green | Green | Green |
| algal growth inhibition- | <i>Pseudokirchneriella subcapitata</i> | 0.2 | AHR | Moderate to negligible | >0.05 to >0.5 | NA | Yellow | Yellow | Yellow | Yellow | Yellow | Green |
| freshwater blue-green alga | <i>Anabaena flosaquua</i> | 39 | NR | Negligible | <0.05 | NA | Green | Green | Green | Green | Green | Green |
| Duckweed | <i>Lemna gibba</i> | 0.0032 | CR to ARU | High to moderate | > 1 to > 0.5 | NA | Red | Red | Red | Red | Red | Yellow |
| Earthworm | <i>Eisenia fetida</i> | 1.25 | ARU to NR | Moderate to negligible | <0.5 to <0.05 | NA | Yellow | Green | Green | Green | Green | Green |
| Soil macro-organisms: Collembola | <i>Folsomia candida</i> | 2.68 | AES to NR | Low risk to negligible | >0.05 to <0.05 | NA | Blue | Blue | Blue | Blue | Green | Green |
| Mammalian acute (Rat) | <i>Rattus norvegicus</i> | 2500# | NR | Negligible | <0.05 | NA | Green | Green | Green | Green | Green | Green |
| Dogs | - | 26.7 | NR | Negligible | <0.05 | NA | Green | Green | Green | Green | Green | Green |

Acute LD₅₀ value were used in absence of NOEC (EFSA 2013)

Level of concern (LOC), 0.5 or above, Acute High Risk (AHR); 0.1 Acute Restricted Use (ARU); 0.05 Acute Endangered Species (AES); 1 Chronic Risk (CR); <0.05, No risk;

| RQ | Risk | RQ | Risk | RQ | Risk | RQ | Risk |
|----|------|------------|----------|--------------|---------------------|-------|------------|
| ≥1 | High | ≥0.1 and 1 | Moderate | ≥ 0.1 - 0.01 | Endurate (Low risk) | <0.01 | Negligible |

The results confirm that chronic dietary exposure to tembotrione, when applied at recommended agricultural doses, poses negligible health risks to humans, remaining well within the ADI threshold. Table 3 also presents RQ values for various taxa, along with corresponding LOC benchmarks defined by the U.S. Environmental Protection Agency (EPA). According to EPA criteria, LOC values exceeding 0.5, 1.0, 0.05, and 1.0 indicate acute high risk, acute restricted use, acute endangered species risk, and chronic risk, respectively. These LOC thresholds are used by regulatory agencies to interpret ecological risk and to determine the need for further regulatory actions.

For aquatic and soil organisms—including fish, algae, and macroflora—risk assessment was conducted using NOEC (No Observed Effect Concentration) or LC_{50}/EC_{50} values, as detailed in Table 3. This approach is consistent with methodologies reported by Vasickova et al. (2019) and Zhou et al. (2019), who also utilized NOEC and LC_{50} values for ecological risk assessments. Additionally, hazard quotient (HQ) values at harvest were found to be below 1, further supporting the conclusion of low risk from dietary exposure. Risk characterization based on the detection of tembotrione residues in soil and water was conducted using risk quotient (RQ) values for representative non-target organisms. For soil macro-organisms, the RQ values for *Folsomia candida* (Collembola) indicated a low to negligible risk up to 90 days post-application. In aquatic environments, *Lemna gibba* (duckweed) exhibited RQ values ranging from high to negligible risk up to 60 days, with a moderate risk persisting at 90 days. The fathead minnow (*Pimephales promelas*), used to evaluate early life stage risk in fish, showed moderate risk up to 20 days, with RQ values declining to non-significant levels thereafter.

For *Pseudokirchneriella subcapitata*, representing algal growth inhibition, RQ values indicated a moderate risk up to 60 days. In the case of *Eisenia fetida* (earthworms), a moderate risk was observed at 5 days post-application, which diminished to negligible levels from day 10 onward. RQ values were consistently negligible for *Oncorhynchus mykiss* (rainbow trout), *Daphnia magna* (water flea), *Lepomis macrochirus* (bluegill sunfish), and *Anabaena flos-aquae* (freshwater blue-green alga).

Regarding mammalian toxicity, *Rattus norvegicus* (rat) and domestic dogs were selected as representative species. For both, RQ values remained negligible across all sampling intervals, indicating minimal chronic risk from environmental exposure to tembotrione residues (Table 3).

3.5 Dietary risk assessment of tembotrione:

Chronic dietary exposure to tembotrione was evaluated using the deterministic approach outlined in WHO guidelines (WHO, 2019; 2020). In accordance with the International Programme on Chemical Safety (IPCS, 2009), such assessments are mandated for all registered crops under evaluation. In India, tembotrione is registered for use on maize, with a maximum residue limit (MRL) established at 0.02 mg/kg (EPA, 2007). Based on mean residue data collected over a three-year period, chronic dietary exposure for humans did not exceed the acceptable daily intake (ADI) on any of the sampling days. Correspondingly, hazard quotient (HQ) values for humans remained below 1 across all sampling intervals, indicating no appreciable risk of adverse health effects from the consumption of maize grains or fish containing tembotrione residues.

Conversely, dietary risk to livestock, particularly cattle, was found to be higher. HQ values exceeded 1 when maize fodder aged 30 to 60 days was considered as animal feed, suggesting a potential health risk during this period. At harvest, however, HQ values were below 1, indicating a low risk of adverse effects if fodder is provided at this stage. Specifically, chronic dietary exposure based on residue levels in 30-day-old maize fodder exceeded the ADI for cattle, while exposures at 60, 90 days, and at harvest remained within acceptable safety margins (Table 4).

TABLE 4

Calculated dietary risk assessment of tembotrione based on ADI and RFD and level of concern on human and animal life based on residues detected in the fish, plant, maize grain and straw samples collected from maize field where tembotrione was applied continuously for three years

| | Parameter | Fish | Maize Grain | Plant | Maize Straw |
|--|-----------|----------------------|----------------------|----------------------|-------------|
| Mean Residues (mg/kg) | 30 days | 0.02 | NA | 0.2 | NA |
| | 60 days | 0.06 | NA | 0.02 | NA |
| | 90 days | 0 | NA | 0.01 | NA |
| | Harvest | 0.001 | 0.001 | 0.007 | 0.008 |
| Rfd (mg/kg) | | 0.00008 | 0.00008 | 0.00008 | 0.00008 |
| Tolerance Limit (mg/L) | | 0.02* | 0.02 | 1 | 0.5 |
| Consumption of Food (kg/day) | | 0.1 | 0.08 | 15 | 15 |
| Chronic Dietary Risk Exposure (mg/kg/day) | 30 days | 2.44E ⁻⁰⁵ | NA | 0.0023 | NA |
| | 60 days | 8.85E ⁻⁰⁵ | NA | 0.0002 | NA |
| | 90 days | 1.54E ⁻⁰⁶ | NA | 0.0001 | NA |
| | Harvest | 2.37E ⁻¹⁰ | 1.23E ⁻⁰⁶ | 8.18E ⁻⁰⁵ | 0.0002 |
| ADI (mg/kg bw/day) | | 0.0004 | 0.0004 | 0.0004 | 0.0004 |
| % ADI Risk | | <ADI | <ADI | >ADI | <ADI |
| Dietary Exposure Index | 30 days | 0.31 | NA | 85 | NA |
| | 60 days | 1.11 | NA | 7.63 | NA |
| | 90 days | 1.54E ⁻⁰⁵ | NA | 4.23 | NA |
| | Harvest | 1.54E ⁻⁰⁵ | 0.00008 | 0.00022 | 3.233 |
| Hazard Quotient | 30 days | 0.25 | NA | 68 | NA |
| | 60 days | 0.89 | NA | 6.1 | NA |
| | 90 days | 0.02 | NA | 3.38 | NA |
| | Harvest | 2.37E ⁻⁰⁶ | 0.012 | 0.818 | 0.862 |

3.6 Correlation among tembotrione physicochemical properties, residues in soil, and weather parameters:

Correlation analysis revealed that tembotrione residues in maize field soil were significantly and positively correlated with initial residue concentrations ($R^2 = 0.84, p = 0.05$), residue levels at 60 days ($R^2 = 0.84, p = 0.05$), and overall persistence ($R^2 = 0.83, p = 0.05$). In contrast, significant negative correlations were observed with degradation percentage at 60 days ($R^2 = -0.83, p = 0.05$), octanol–water partition coefficient ($Pow, 24\text{ }^\circ\text{C}; R^2 = -0.81, p = 0.05$), total rainfall ($R^2 = -0.97, p = 0.05$), and number of rainy days ($R^2 = -0.805, p = 0.05$). Other factors, including vapor pressure (Torr), temperature, water solubility (g/L), humidity, evaporation rate, and dissociation constant (pKa), showed no significant correlation with initial residue levels.

Tembotrione degradation in soil was positively correlated with Pow ($R^2 = 1.00, p = 0.05$) and humidity ($R^2 = -0.86, p = 0.05$), and negatively correlated with herbicide water solubility ($R^2 = -0.879, p = 0.05$) and evaporation ($R^2 = -0.879, p = 0.05$). No significant correlations were observed for vapor pressure, temperature, rainy days, or pKa in relation to degradation rates. The half-life of tembotrione in soil exhibited positive correlations with persistence at 60 days ($R^2 = 0.87, p = 0.05$), water solubility ($R^2 = 1.00, p = 0.05$), and evaporation ($R^2 = 0.99, p = 0.05$), indicating moderate environmental stability. Conversely, half-life was negatively correlated with degradation at 60 days ($R^2 = -0.88, p = 0.05$), average maximum temperature ($R^2 = -0.87, p = 0.05$), humidity ($R^2 = -1.00, p = 0.05$), and Pow ($R^2 = -0.89, p = 0.05$). Other physicochemical properties, including vapor pressure, temperature, evaporation rate, and pKa, showed no significant influence on half-life (Table 5).

TABLE 5

Pearson correlation matrix for various soil and herbicides parameters describing degradation of tembotrione in soil of maize field

| | 1. | 2. | 3. | 4. | 5. | 6. | 7. | 8. | 9. | 10. | 11. | 12. | 13. | 14. | 15. | 16. |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-----|-------|------|-----|-------|-----|
| year | 1 | | | | | | | | | | | | | | | |
| Initial Residues | -1 | 1 | | | | | | | | | | | | | | |
| Residues at 60 days ($\mu\text{g g}^{-1}$) | -0.88 | 0.84 | 1 | | | | | | | | | | | | | |
| Av. Half-life (days) | -0.52 | 0.45 | 0.86 | 1 | | | | | | | | | | | | |
| Persistence at 60 day | -0.87 | 0.83 | 1 | 0.87 | 1 | | | | | | | | | | | |
| Degradation at 60 day (%) | 0.87 | -0.83 | -1 | -0.88 | -1 | 1 | | | | | | | | | | |
| Total rainfall | 0.94 | -0.97 | -0.67 | -0.21 | -0.66 | 0.66 | 1 | | | | | | | | | |
| Av max Temperature | -0.3 | 0.37 | -0.19 | -0.66 | -0.21 | 0.21 | -0.6 | 1 | | | | | | | | |
| Av max Temperature | 0.04 | 0.04 | -0.51 | -0.87 | -0.53 | 0.53 | -0.29 | 0.94 | 1 | | | | | | | |
| Herbicide water solubility (mg/L) | -0.5 | 0.43 | 0.85 | 1 | 0.86 | -0.86 | 0.18 | -0.67 | -0.89 | 1 | | | | | | |
| vapor pressure (Torr, 20 °C) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | | | | | |
| Humidity (%) | 0.5 | -0.43 | -0.85 | -1 | -0.86 | 0.86 | 0.18 | 0.67 | 0.89 | -1 | 0 | 1 | | | | |
| Av number of rainy days | 0.8 | -0.85 | -0.42 | 0.09 | -0.4 | 0.4 | 0.96 | -0.81 | -0.56 | 0.11 | 0 | -0.12 | 1 | | | |
| Dissociation constant (pKa) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | | |
| Octanol water partition coefficient (Pow 24 °C) | 0.85 | -0.81 | -1 | -0.89 | -1 | 1 | 0.63 | 0.24 | 0.56 | -0.88 | 0 | 0.88 | 0.37 | 0 | 1 | |
| Evaporation (mm) | -0.38 | 0.31 | 0.78 | 0.99 | 0.79 | -0.79 | -0.05 | -0.77 | -0.94 | 0.99 | 0 | -0.99 | 0.24 | 0 | -0.81 | 1 |

Tembotrione dissipation in both soil and maize plants followed first-order kinetic models. The calculated DT_{50} (half-life) values ranged from 12.57 to 15.67 days in soil and from 9.25 to 12.57 days in maize plants, with mean half-lives of 14.0 and 11.4 days, respectively. Faster degradation rates were observed in the second and third years of the study, likely influenced by climatic and edaphic factors (Figure 4).

Similar dissipation behavior was reported by Zhang et al. (2015), who observed first-order kinetics for tembotrione in low pH Red-Yellow Latosol soils, although with substantially longer half-lives ranging from 61 to 90 days. In contrast, Silva et al. (2019) documented significantly higher persistence of tembotrione in medium- and clay-textured soils, with detectable residues persisting for up to 200 days in humid conditions and 300 days under water-deficit conditions. These findings highlight the influence of soil properties and moisture regimes on tembotrione persistence.

Elevated half-life values, such as those observed in our study, may indicate a potential carryover risk, as previously reported by Zhang et al. (2015). The interaction between sorption and degradation is well-established for other triketone herbicides, including mesotrione. Mendes et al. (2017) noted that rapid degradation in soil can be constrained by the adsorption capacity of soil particles, which limits bioavailability.

Austria (2011) reported 105 days for 90% dissipation of tembotrione under field conditions, while laboratory studies by PMRA (2014) indicated persistence beyond 342 days, underscoring the variability in degradation depending on environmental conditions. Similarly, slow degradation in Vertisol soils was documented by Zemelka (2015). Prolonged persistence has been linked to phytotoxic effects in subsequent crops, including sugar beet (Silva et al., 2019), potato (Dias et al., 2019), and carrot (Bontempo et al., 2016).

Conversely, rapid degradation in maize fields was reported by Sue et al. (2020), who found tembotrione residues in maize grain and corncob matrices to be below 0.02 mg/kg, consistent with the findings of our study regarding low residue levels at harvest.

The highest tembotrione half-life in the soil of maize field was found in 2017 when compared to that observed in the same soil with other years; this can be correlated with the physicochemical properties of the soil as well as weather conditions prevailed during the experimental period, especially high rainfall and humidity that might have contributed to more microorganism degradation in 2018 and 2019 (Zhang et al., 2015; Sondhia et al., 2016). A direct correlation between soil clay content, soil organic matter and half-life was reported by Silva et al. (2019). Lower half-life of tembotrione is reported at high pH soil due

to a decrease in the number of cationic molecules present in the soil (Trigo et al., 2014). Since tembotrione is a weak acid (pKa, 3.2) and is negatively charged in solution, hence, it is repulsed in those circumstances where soil pH is increased and inhibits microbial degradation.

In this study, less quantity of tembotrione residues were detected in the pond water samples than in maize field soil. However, detection of tembotrione residues at 30 and 60 days in fish limits its consumption at these days. However, in this study, tembotrione residues in fish were not detected (<0.001 mg/kg) after 90 and 100 days. The hazard quotient at all sampling days was also found to be <1 for humans and indicates that there is no appreciable risk of adverse health effects following dietary exposure to tembotrione through consumption of fish or maize grains. Negligible dietary risk after tembotrione application in maize field was also reported by Su et al. (2020) and Cao et al. (2022). However, due to tembotrione residues in the pond and fish up to 60 days, an appropriate remediation procedure is required to limit its release into neighboring water systems. Similarly, due to relatively high to moderate RQ of tembotrione toxicity to aquatics, such as fathead minnow and duckweed, the release of maize herbicides through runoff into receiving water canals should be minimized through appropriate measures.

Rainfall and temperature variations played a significant role in the degradation dynamics of tembotrione in maize field soil over the three-year study period. Lower cumulative rainfall totals in 2017 and 2018 (967.1 mm and 1092.1 mm, respectively) contrasted with the substantially higher precipitation recorded in 2019 (1604.8 mm), likely facilitating the more rapid degradation of tembotrione observed during the third year. Correspondingly, tembotrione residue concentrations at 20 days post-application were higher in 2017 (0.224 mg/kg) and 2018 (0.193 mg/kg) compared to 2019 (0.093 mg/kg), suggesting that increased rainfall contributed to enhanced dissipation in the latter year.

While elevated rainfall may accelerate tembotrione degradation in soil, it simultaneously increases the potential for herbicide runoff into adjacent aquatic ecosystems, raising concerns regarding off-site contamination during the maize cropping season (Sondhia et al., 2013; 2016). Although tembotrione dissipation consistently followed first-order kinetics each year, degradation rates in both soil and maize plants exhibited inter-annual variability, likely driven by fluctuating environmental conditions.

IV. CONCLUSION

This study provides a comprehensive evaluation of the long-term environmental and health behavior of tembotrione, a triketone herbicide widely used in maize cultivation. Under field conditions, tembotrione demonstrated rapid soil dissipation, indicating low persistence in subtropical agroecosystems. Human dietary exposure through maize grains and fish consumption consistently yielded health risk quotients below one across all sampling intervals, suggesting minimal risk. In contrast, a potential dietary risk to livestock, particularly cattle, was identified when maize plants were harvested 30 to 60 days post-application, with risk quotients exceeding one. Additionally, aquatic organisms may be temporarily affected by runoff shortly after application. While soil macro-organisms exhibited a wide range of risk levels over time, from high to negligible, this risk is mitigated by the herbicide's low volatility and limited environmental stability. Health risk assessments revealed varying degrees of concern for animals, but negligible risk for humans. Overall, the findings support the safe use of tembotrione in maize production when integrated with appropriate risk management strategies, particularly concerning livestock feeding schedules and mitigation of aquatic exposure.

CONFLICTS OF INTEREST

The authors declare no known competing financial or personal interest in the work of this paper.

DATA AVAILABILITY STATEMENT

All data are found within the main manuscript and original sources as stated

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