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Detection and Screening of Organic Contaminants in A Riverine System of Georgia Using Non-Targeted Analysis

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Abstract: Numerous organic chemicals exist within aquatic environments, yet effectively screening and prioritizing them is a huge challenge. This study provides a comprehensive investigation into the ecological dynamics of the North Oconee River within Athens-Clarke County, Georgia, with a specific focus on the distribution of 33 identified compounds, including a prominent pesticide. The research, conducted in the riverine ecosystems proximal to the Firefly trail, employs advanced analytical techniques to elucidate potential contamination sources arising from agricultural and urban runoff. Intriguingly, the study reveals North Oconee River near the Firefly Trail as a notable site for heightened pesticide contamination, warranting a meticulous exploration of its origins. Furthermore, the investigation unveils the intricate microbial degradation processes of malathion within the North Oconee River, elucidating the pivotal role played by microbial activity in river water. The detection of degradant byproducts prompts the considerations of bioavailability and toxicity, associating potential implications for the river's overall ecological health. Ongoing research endeavors to precisely quantify environmental risks and unravel indigenous microbial degradation pathways, presenting pivotal contributions to the scientific community's understanding of complex riverine ecosystems. This research serves as a foundational piece in informing sustainable environmental management practices and emphasizes the urgency of comprehensive stewardship in safeguarding aquatic ecosystems.

Keywords: pesticide distribution; riverine ecosystems; biotransformation; toxicity; environmental health

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1. Introduction

Since the advent of large-scale commercial production of anthropogenic chemicals, an extensive array of harmful organic compounds has been introduced into the aquatic environment, either directly or indirectly [1–3]. Despite many of these compounds existing at relatively low concentrations, measured in nanograms per liter or per gram, their persistence and bio-accumulative nature render them potent threats to aquatic ecosystems [4–6]. This persistence can lead to acute or chronic toxicity in aquatic organisms, posing significant ecological risks.

Remarkably, only a fraction of these toxic organic compounds is currently subject to monitoring and regulation [7]. Given the sheer diversity and potential impact of these compounds, it is imperative to establish a comprehensive understanding of their presence and behavior in aquatic environments [3]. Such an overview is essential for identifying compounds that may degrade naturally over time and those that persist, allowing for targeted regulatory measures and mitigation strategies.

The complex interplay between organic pollutants and aquatic ecosystems underscores the urgency of adopting proactive approaches to safeguard water quality and biodiversity. Failure to adequately address these challenges could exacerbate the already precarious state of global water resources, with far-reaching implications for both ecosystems and human well-being. Therefore, concerted efforts are needed to expand monitoring and regulation frameworks, alongside investments in research aimed at elucidating the behavior and impacts of organic compounds in aquatic environments. By doing so, we can work towards a more sustainable and resilient future for our planet's water ecosystems.

Pesticides play a significant role in global agricultural practices, including their extensive use in the United States. These chemical substances are employed to eliminate or control pests that can adversely affect crop yields and quality [1,2]. The application of pesticides is a common strategy to ensure the productivity and sustainability of agricultural production worldwide. In the context of the United States, pesticides are widely utilized in both conventional and modern agricultural systems. The application of pesticides in the U.S. is regulated by government agencies, including the Environmental Protection Agency (EPA), which sets guidelines and standards to ensure the safe and responsible use of these chemicals (USEPA: Regulatory Guidance Information). The use of pesticides in the United States is diverse, ranging from large-scale industrial agriculture to smaller, localized farming operations. Different types of pesticides, such as insecticides, herbicides, and fungicides, are employed based on specific pest management needs [1,8]. The choice of pesticides depends on factors such as the type of crops grown, the prevailing environmental conditions, and the targeted pests.

While pesticides contribute significantly to increased agricultural productivity, their use has raised environmental and health concerns. The runoff of pesticides into water bodies, soil contamination, and potential harm to non-target organisms are some of the challenges associated with pesticide application. Therefore, there is an ongoing effort to strike a balance between the benefits of pest control and the potential adverse effects of pesticide use, emphasizing sustainable and environmentally friendly practices in agriculture.

The current research undertakes a thorough investigation into the distribution and destiny of organic pesticides and associated compounds within the North Oconee River watershed situated in Athens-Clarke County, Georgia, USA. The specific aim of this study has two open questions:

1. What specific organic contaminants are prevalent in the water of the North Oconee River watershed in Athens-Clarke County, Georgia, given its significance as a drinking water source?
2. Are chemical and biological transformations occurring within the North Oconee River watershed system, potentially altering the composition or toxicity of organic pesticides and related compounds?

The North Oconee River watershed encompasses an area of 311 mi^2, with 94% of its upper section situated north of Athens-Clarke County [9]. Within Athens-Clarke County, 20 mi^2 belong to the watershed [10,11]. Flowing south through the heart of Athens, including the downtown area, the river runs along the eastern edge of the University of Georgia campus, extending south from River Road. In terms of land cover, the study area predominantly features developed land and forests, with approximately 15% impervious cover [12].

2. Materials and Method

Conducting a comprehensive assessment of the organic compound levels in the North Oconee River, Georgia, we focused on a particular sampling site—Firefly Trail (FT) in Athens (Figure 1). This site was strategically selected due to its proximity to residential and industrial areas, making it susceptible to urban runoff. The study spanned five sampling events from August 2023 to January 2024 (Table 1).

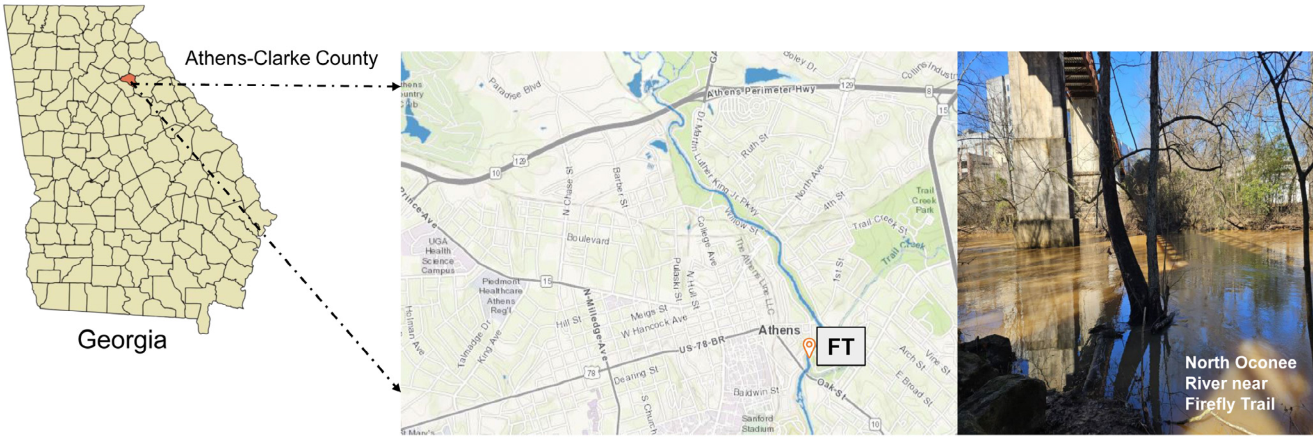


Figure 1. Overview map of the study area and sampling sites, featuring representative images of Firefly Trail (FT), located in Athens, Georgia, USA.

Table 1. Details of sampling locations and date of collection with physicochemical parameters measured on the field.

Sample Site	Date	Latitude and Longitude	Temp (°C)	pH	ORP	DO (ppm)	Conductivity (µS/cm)	TDS (ppm)	Salinity (PSU)	Turbidity (NTU)
Firefly Trail (FT)	08/25/2023 (E*1)	33.956652 N, 83.367263 W	31.2	7.19	212	2.3	179	78	0.005	26.5
	09/19/2023 (E2)		30.8	7.2	218.8	3.1	114	73	0.004	27.1
	10/19/2023 (E3)		13.87	7.26	159.4	4.8	156	69	0.08	31.4
	12/12/2023 (E4)		8.1	6.9	190	2.9	120	101	0.08	25
	30/1/2024 (E5)		7.3	6.43	179.2	6.1	100	45	0.05	29

* Note: All sampling events are denoted as "E".

2.1. Sample Collection and Preservation

Water samples were collected using a telescopic sampler, consisting of an HDPE beaker connected to a stainless-steel rod, allowing for sampling at greater distances. The beaker was carefully rinsed onsite at the sampling location. The water samples (500 mL) were collected in a pre-baked (450 °C for 4 h) 1 L glass amber bottle for chemical analysis and in a clean and sterilized 500 mL HDPE bottle, which had been previously autoclaved at 121 °C for 15 min, for the microbial degradation study.

Water parameters, including pH, dissolved oxygen, oxidation–reduction potential (ORP), conductivity, total dissolved solids (TDS), turbidity, and salinity, were measured using the HANNA HI9829 probe (Hannah Instruments, Smithfield, RI, USA) (Table 1). The probe was calibrated using the quick calibration solution before recording the parameters into the database. To preserve the samples until further analysis, they were stored at 4 °C in a refrigerator for 24 h.

2.2. Extraction of Water Samples

Water sample extraction involved the use of solid phase extraction (SPE) to extract compounds for subsequent non-targeted and targeted analysis. Before moving forward with the extraction procedure, the samples were filtered with a 0.45 µm nylon-66 filter paper (47 mm dia.) followed by the injection of 20 µL of naphthalene-d8 (Millipore Sigma®, Burlington, VT, USA) as an internal standard. The samples were extracted through OASIS® HLB (Hydrophilic–Lipophilic Balance) cartridges (6 cc Vac Cartridge, 200 mg sorbent per Cartridge; Waters, Milford, MA, USA), which were pre-conditioned with 5 mL of methanol (GCMS SupraSolv®, Supelco, Bellefonte, PA, USA). Cartridges were washed with 5 mL Type 1 ultrapure water three consecutive times. Extracts were then eluted using 5 mL of acetone (Optima™, Fisher Chemical™, Waltham, MA, USA) and 5 mL of hexane (GCMS SupraSolv®, Supelco, Bellefonte, PA, USA). The eluent was transferred into a clean test tube containing 5 g of sodium sulfate anhydrous to absorb excess moisture in the extract. The supernatant was concentrated to 200 μL using controlled nitrogen purging. Thereafter, 20 μL of the extracts were taken in a 2 mL GC amber vial and reconstituted with 100% hexane up to 1 mL. The samples were then stored in a −20 °C freezer until analysis (Figure S1). The extraction procedure was modified from Duttagupta et al. [1].

2.3. Microbial Degradation Study of Malathion in Different Environmental Conditions

Water samples obtained during the October event from the Firefly trail were utilized to evaluate the microbial degradation of the predominant pesticide under various environmental conditions (Table S1 in Supplementary Material). Malathion emerged as one of the most prevalent and frequently detected pesticides in this study. To facilitate the investigation, commercial grade Spectracide®, Middleton, WI, USA malathion insect spray was employed, given its widespread use globally across agricultural, gardening, and household applications. Each experimental set comprised 100 mL of sample spiked with 0.2% commercial grade malathion, maintained at 30 °C on a shaker operating at 100 rpm for 24 h of incubation.

2.4. Instrumental Analysis

Non-targeted analysis was conducted using 8860 GC, 5977B MS Gas Chromatography–Mass Spectrometry (Agilent Technologies®, Santa Clara, CA, USA) in full scan mode for the non-targeted qualitative approach. The targeted analysis was also conducted with the same sample extract for malathion as the primary analyte in SIM mode with m/z 93.0, 125.0, and 173.0. Detailed instrumentation conditions can be found in Table 2. The external calibration curves consisted of six standard solutions ranging from 0.1 to 500 ng/mL of malathion (PESTANAL®, Supelco, Bellefonte, PA, USA) with an isotope-labeled internal standard, naphthalene-d8.

Table 2. GC/MS conditions for non-targeted and targeted screening in Scan and SIM mode.

GC	
Agilent 8860 GC System with Auto-Injector and Tray	
Inlet	
	Split/Splitless inlet
Mode	Pulsed splitless
Injection pulse pressure	50 psi until 0.7 min
Purge flow to split vent	50 psi until 0.75 min
Injection volume	1 μL
Inlet temperature	280 °C
Carrier gas	Helium
Inlet liner	Agilent low pressure-drop (LPD) with glass wool
Oven	
Initial oven temperature	80 °C
Initial oven hold	1.5 min
Ramp rate 1	40 °C/min
Final temperature 1	120 °C
Final hold 1	0 min
Ramp rate 2	10 °C/min
Final temperature 2	300 °C
Final hold 2	4 min
Total run time	24.5 min

Table 2. *Cont.*

Post run time	1.5 min
Equilibration time	0.25 min
Column	
Type	Agilent J&W HP-5 ms Ultra Inert
Length	30 m
Diameter	0.25 mm
Film thickness	0.25 µm
Control mode	Constant flow
Flow	1.374 mL/min
Inlet connection	Split/Splitless
Outlet connection	MSD
MSD	
Model	Agilent 5977B MSD
Tune file	CUSTOM.U
Mode	Scan and SIM
Scan range	45 to 550 amu
Solvent delay	4 min
Quad temperature	150 °C
Source temperature	280 °C
Transfer line temperature	280 °C
SIM (m/z)	93.0, 125.0, 173.0

2.5. Non-Targeted Data Analysis

The National Institute of Standards and Technology (NIST) Mass Spectral Library was used for peak identification. A list of compounds is compiled for each sample type using the following criteria:

- Found in all the samples collected during a sampling event.
- Not found in any of the field blanks or, if found in a field blank, the peak area should be three times larger in the samples.
- Has a similarity score (as calculated by the Mass Hunter qualitative software, v 10.0, Agilent Technologies®, USA) to a reference spectrum in the NIST library of ≥500.
- Three or more molecular ions in the sample should match with the NIST library to hit a peak for a given analyte.

Compounds that meet the first two criteria only are herein referred to as “qualified” compounds. To correct instrumental variability, peak area normalization is performed. This involves adjusting the sample peak areas using an internal standard (naphthalene-d8). The peak area of each compound is divided by the peak area of naphthalene-d8 to obtain the normalized peak area. A median value of the normalized peak areas of all samples for each compound is determined and used for abundance comparison. No quantitative data (i.e., concentration) are collected during this method, but relative abundance can be used to compare compound abundance within the samples.

Heatmap and cluster analyses were carried out with the heatmap package [13] and based on the peak response of best-hit compounds across different sampling time points. Column clustering, which displayed the clustering of different samples, was based on Euclidean distance. Each row (depicting the peak response of the organic compound) was normalized using feature scaling and clustered based on correlation.

2.6. Quality Assurance and Quality Control (QA/QC)

All sample analyses were conducted in duplicates to ensure accuracy and reliability. The average recovery rate of the internal standard was determined to be 86.5%. The limit of quantification (LOQ) for malathion was established at 0.1 ng/mL. To standardize the measurements, the concentration of malathion in the water samples was converted to ng/L based on water volume. Prior to extraction, all the glassware used underwent pre-cleaning and was subjected to baking at 450 °C for 4 h. Extracted water samples were promptly stored at −20 °C until analysis. In the microbial degradation study, each sample set was processed in duplicates to validate the consistency of experimental outcomes. Ultra-purified type 1 water (ELGA® water purifying system, Woodridge, IL, USA) served as the laboratory blank for reference. To ensure the quality of the analysis, field blanks with type 1 ultrapure water were also collected in each sampling event, similarly, in a pre-baked 1 L amber glass bottle.

3. Results and Discussion

3.1. Physiological Parameter Dynamics in River Water

Eight physiological parameters were consistently monitored during each sampling event, encompassing temperature, pH, oxidation–reduction potential (ORP), dissolved oxygen (DO) measured in parts per million (ppm), conductivity (µS/cm), total dissolved solids (TDS) in ppm, salinity (PSU), and turbidity (NTU) (Table 1). Analysis of the collected data revealed notable patterns across different seasons. During the dry period observed in the August (8/25) and September (9/19) sampling events, elevated temperatures were recorded alongside lower levels of dissolved oxygen. Conversely, during the winter months, a contrasting trend emerged. As temperatures dropped to their lowest points, there was a notable rise in dissolved oxygen levels, except for an anomaly in December. It is likely that total dissolved solids, possibly from surface run-off, contributed to the decrease in dissolved oxygen during that event. This phenomenon suggests a favorable water flow dynamic during winter, contributing to improved oxygenation levels. However, it is noteworthy that the ORP exhibited a consistent trend, showing slightly higher values during the summer months across all sampling events. This suggests a prevailing oxidative environment during the warmer seasons. Additionally, conductivity levels were observed to be higher during the summer months at both sampling locations.

3.2. Extent of Organic Contaminants Detected in River Water

A diverse array of compounds was identified using non-targeted analysis of water samples across all sampling events. In total, 206 compounds were detected, with some overlapping between different sites. However, 33 compounds met the screening criteria (mentioned in Section 2.5) and are considered high confidence matches. These include esters, ketones, aldehydes, organophosphates, organochlorides, polycyclic aromatic hydrocarbons (PAHs), esters, plasticizers heterocyclic compounds, and others (Table 3, Figure S2). The detection of pesticides and herbicides, including malathion, aldrin, endrin, heptachlor, and methoxychlor, within the North Oconee River watershed implies potential contamination originating from agricultural and urban runoff, given the widespread utilization of these compounds for pest and weed control in agriculture [14]. The presence of these pesticides in the river water raises concerns about potential ecological and environmental impacts. Their persistence in nature and bioavailability may have lasting effects on the overall biodiversity of the water body. Additionally, PAHs such as naphthalene and fluorene were also detected. The continuous presence of triacetin in all sampling events suggests a significant impact of stormwater runoff in Athens-Clarke County and influence from industrial and agricultural activities on the North Oconee River. This array of compounds emphasizes the need for comprehensive assessment and management strategies to address potential environmental implications in the river ecosystem. Figure 2 illustrates the most abundant compounds detected through non-targeted analysis, with malathion consistently standing out as the most abundant across all sampling events for both locations. Average peak areas indicate that malathion was highly abundant and frequent, followed by naphthalene, benzophenone, triacetin, malaoxon, 4-methyl-2,6,7-trithiabicyclo[2.2.2.] octane, aldrin, tinuvin 234, endrin, and heptachlor in FT.

Table 3. List of compounds with high confidence detected consistently throughout all sampling events along the Firefly Trail (FT), along with their respective chemical categories.

Chemical Categories	Name of the Analyte
Organophosphates	Malathion Malaoxon Phosphorodithioic acid, O, O, S-trimethyl ester (also can be categorized under ester) Succinic acid, mercapto-, diethyl ester, S-ester with O, S-dimethyl phosphorodithioate
Polycyclic Aromatic Hydrocarbons (PAHs)	Naphthalene Fluorene 9H-Fluorene, 9-methylene-
Organohalides	Aldrin Methoxychlor Heptachlor Benzaldehyde, 3,4-dimethyl- Endrin Benzenamine, 4-fluoro-2-(trifluoromethyl)-
UV Absorbers	Tinuvin 234 Benzophenone
Plasticizers	Triacetin
Boryl Compounds	Benzaldimine, N-(diethylboryl)-
Acrylates	Diethyleneglycol diacrylate
Esters	Pentanedioic acid, 3-thioxo-, diethyl ester 9-Octadecenoic acid, ethyl ester Diethyl fumarate 2-Butanedioic acid(Z)-,diethyl ester Ethyl 3,3-diethoxypropionate
Aliphatic Compounds	Decane, 2,4-dimethyl- 4-Methyl-2,6,7-trithiabicyclo[2.2.2]octane 2,6,8-Trimethyl-4-nonyl acetate Cetene
Amino Acids and Derivatives	L-Alanine, TBDMS derivative
Thiazole Derivatives	Thiazole-5-methanol, TMS Pyrazole, 5-methyl-3,4-dinitro-1-(piperidinoylmethyl)-
Ketones	4-Heptanone, 2-methyl-
Pyrimidine	2,4,5-Trihydroxypyrimidine
Carboxyl acid amide	Acetamide, N-(2,4-difluorophenyl)-2-[(4,5-dihydro-2-thiazolyl) thio]-

Following the non-targeted analysis, malathion was selected for targeted quantification and further investigation to observe its degradation pathway. Organophosphate pesticides, like malathion, are extensively utilized in the global agricultural sector [15]. Malathion, a pesticide widely employed to combat insects on various fronts—be it crops, stored products, golf courses, home gardens, or outdoor spaces with trees and shrubs—also serves as an agent for mosquito and Mediterranean fruit fly (medfly) control in expansive outdoor areas. Additionally, malathion finds application in flea eradication on pets and the treatment of human head lice. However, less than 1% of malathion reaches pests, resulting in significant environmental discharge and potential soil and water contamination [16]. As per the USEPA guidelines, malathion concentrations within drinking water are considered acceptable at the levels of 0.2 mg/L for both single-day and extended exposure periods in children and 0.1 mg/L for lifelong exposure in adults. Malathion's half-life varies between 2 and 18 days, contingent upon environmental conditions such as temperature and pH levels. Malaoxon, an oxidative metabolite of malathion, exhibits a half-life of 32 days. These findings are essential for assessing the potential health risks associated with malathion exposure to drinking water. Concerns about malathion's adverse impact on human health arise from several factors, including the following: its lipophilic nature reduces solubility in water; it demonstrates a high tendency to accumulate in the environment; and its physical and chemical properties contribute to persistence [17]. The detrimental health effects of malathion stem from the inhibition of the enzyme acetylcholinesterase, leading to documented impacts on internal organs such as the heart and liver, as well as genetic and chromosomal disorders [1,18].

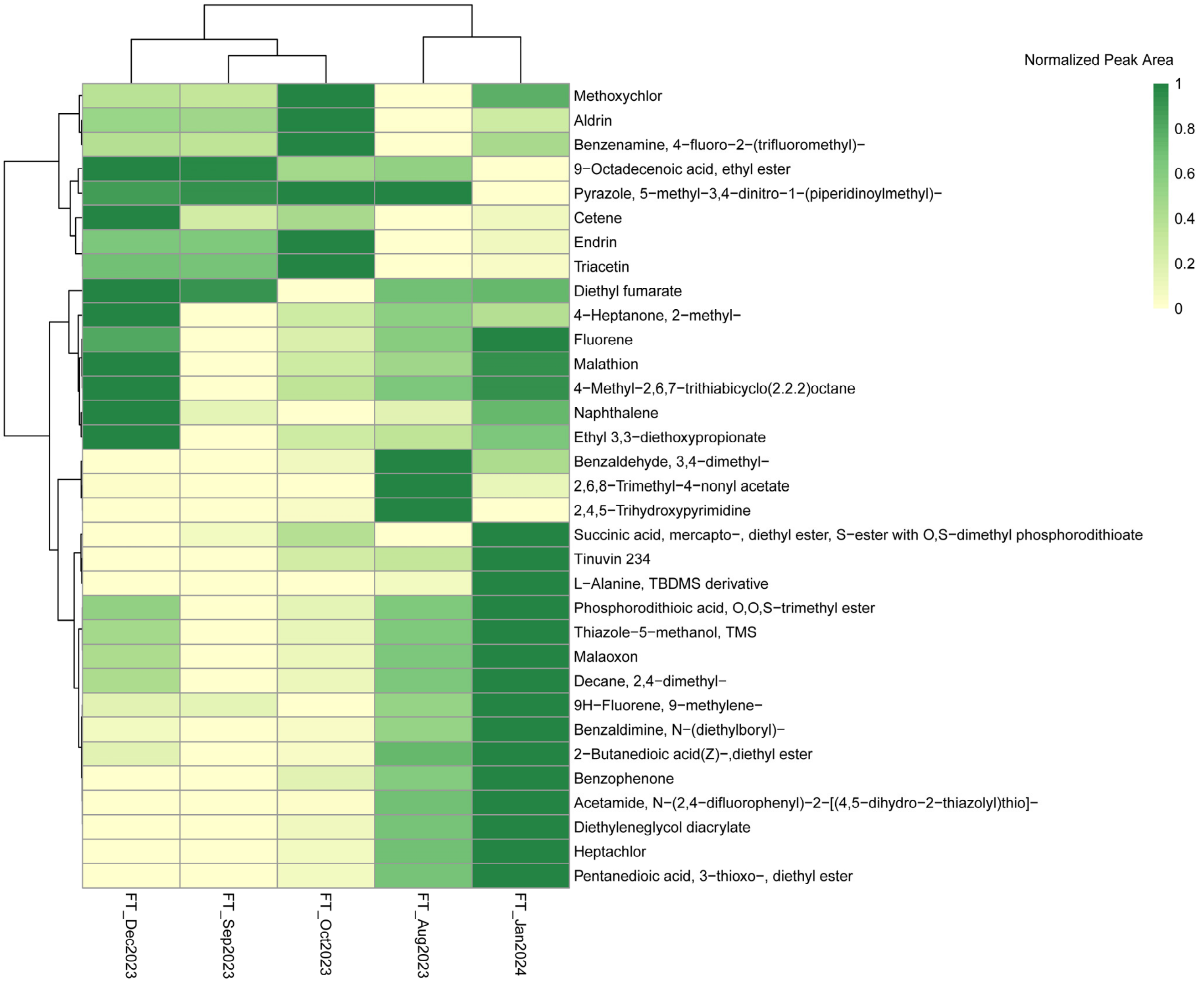


Figure 2. Heatmap illustrating the normalized responses of the identified organic compounds at the FT across all sampling events.

From August 2023 to January 2024, the five sampling events exhibited an upward trend in malathion concentration. The precipitation during the December and January sampling events notably influenced this trend, leading to a 34.2% increase in concentration in December and a subsequent 53.6% increase in January compared to the initial event in August (Figure 3, Table 4). Precipitation can play a significant role in transporting pollutants, including pesticides like malathion, from surrounding areas into water bodies. During rain events, water runoff from agricultural fields, urban areas, or other locations where malathion is used can carry pesticide into rivers or streams, influencing the water quality. It is important to consider the specific local conditions, land use, and weather patterns when interpreting variations in pesticide concentrations in water bodies. Additionally, the findings may warrant further investigation into the specific sources and mechanisms influencing pesticide transport and concentrations in the studied areas.

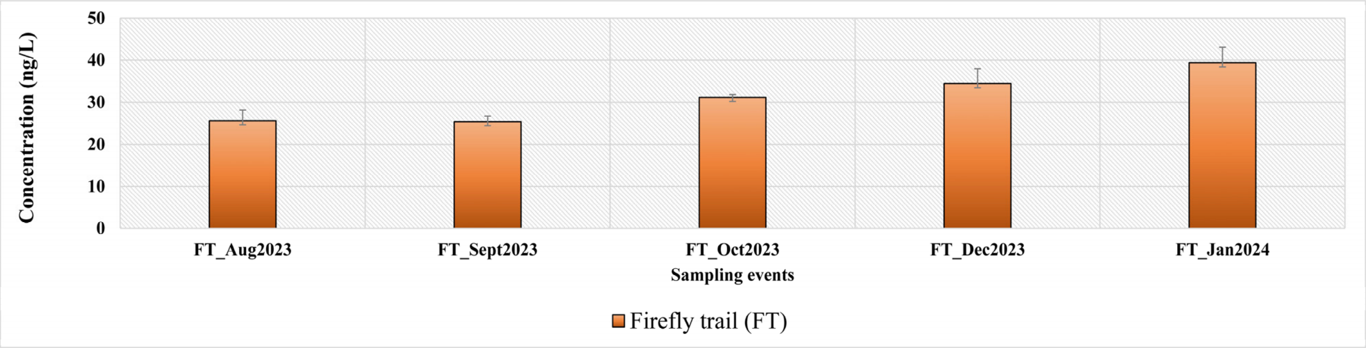


Figure 3. Graph illustrating malathion concentration (ng/L) across five sampling events at FT.

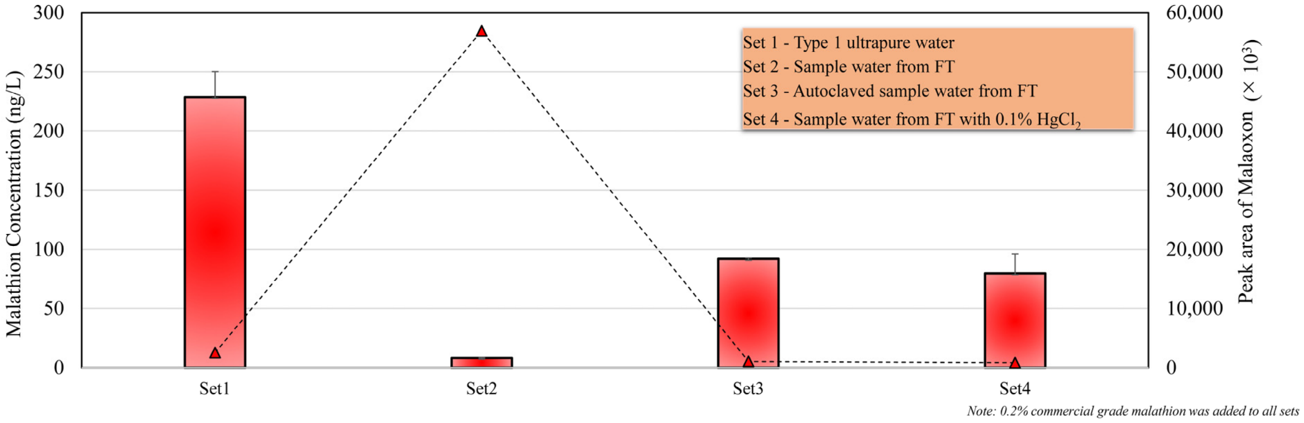
Table 4. Summary of malathion concentrations (ng/L), including descriptive statistics.

Sampling Events	Firefly Trail (FT)
FT_Aug2023 (E1)	25.640
FT_Sept2023 (E2)	24.438
FT_Oct2023 (E3)	31.208
FT_Dec2023 (E4)	34.424
FT_Jan2024 (E5)	39.408
Parameters	**Firefly Trail (FT)**
Min	24.438
Max	39.408
Geomean	30.531
Median	31.208
1st Quartile	25.640
3rd Quartile	34.424
Std Dev	5.554
Average	31.024

3.3. Chemical and Biological Degradation of Malathion

Upon introduction into the environment, typically through crop spraying or broad applications in urban and residential settings, airborne malathion droplets settle on soil, plants, water, or man-made surfaces. While the majority of malathion tends to remain localized, a portion may disperse to areas beyond the application site through surface runoff. Although malathion persists in the environment for periods ranging from a few days to several months, its breakdown typically occurs within 2–3 weeks. Degradation processes involve interactions with water, sunlight, and microbial consortium present in soil and water. Malathion exhibits low soil adherence and undergoes rapid microbial breakdown, minimizing the likelihood of significant groundwater contamination. In water, the compound undergoes swift decomposition facilitated by water and aquatic microorganisms. In the presence of sunlight, malathion breaks down a more toxic byproduct known as malaoxon [1]. In the present study, the non-targeted screening also detected malaoxon in both locations with a higher abundance in FT. To examine the biodegradation of malathion, our attention was directed towards the water samples obtained from the FT sampling site. The objective was to investigate the degradation of malathion and ascertain whether this process leads to the production of the more toxic byproduct, malaoxon.

Based on the laboratory experiments conducted under various environmental conditions, it is apparent that Malathion degradation occurred in the sample water, possibly due to bacterial activity (Figure 4). The experiment comprised four sets with distinct conditions. Notably, the sample water spiked with 0.2% malathion (Set 2) exhibited the lowest concentration compared to all other setups. Furthermore, to inhibit microbial activity, one set involved autoclaving the sample river water and spiking it with 0.2% malathion (Set 3), while another set included spiking with $HgCl_2$ (resulting in an end concentration of 0.1%) and 0.2% malathion (Set 4).



Note: 0.2% commercial grade malathion was added to all sets

Figure 4. Graph depicting malathion concentration (ng/L) (plotted in the bar graph) and peak areas ($\times 10^3$) of malaoxon (plotted in line graph) in four experimental sets. Set 1 includes type 1 ultrapure water spiked with 0.2% malathion, set 2 consists of river water from FT spiked with 0.2% malathion, set 3 features autoclaved river water from FT spiked with 0.2% malathion, and set 4 comprises river water from FT spiked with $HgCl_2$ (resulting final concentration of 0.1%) and 0.2% malathion.

The concentration hierarchy among these environmental sets can be summarized as follows: Set 2 (with an average of 8.11 ng/L ± 0.29) < Set 4 (with an average of 79.66 ng/L ± 8.98) < Set 3 (with an average of 91.73 ng/L ± 1.22) < Set 1 (with an average of 228.58 ng/L ± 30.49) (Figure 4). This ordering highlights the impact of environmental and microbiological degradation factors and treatments on malathion concentration in water samples.

The higher degradation of malathion in river water compared to river water with $HgCl_2$, autoclaved river water, and Type 1 ultrapure water can be attributed to the presence of microbial activity in the untreated river water. Autoclaving involves subjecting a sample to high temperatures and pressure to sterilize it, effectively eliminating microorganisms, including bacteria, which may otherwise contribute to the degradation of pesticides. The addition of 0.1% $HgCl_2$ had a similar impact on malathion degradations in the original river water sample compared to the autoclaved river water sample. This suggests that both methods for inhibiting microbial activity do not have a significant impact on the chemical composition of water. The lowest degradation of malathion was observed in Type 1 ultrapure water. Type 1 ultrapure water typically lacks the microbial and chemical content present in natural water bodies, explaining its lower degradation compared to river water. Comparing all the microcosm setups, chemical and biological transformations are evident in the riverine ecosystem.

Remarkably, the peak areas corresponding to malaoxon suggest that microbial transformation is mainly responsible for the conversion of malathion to malaoxon. The highest peak area for malaoxon was observed in river water collected from FT, followed by Set 4, Set 3, and, lastly, Set 1. This observation suggests the presence of environmental and microbial degradation of malathion, leading to its conversion into malaoxon. The most possible key phenomena contributing to this transformation is bacterial transformation. Given the rapid transformation of malathion in river water, it is more plausible that both chemical and bacterial transformation are occurring [19–21]. Except for Set 2, the other three sets detected malaoxon, albeit in significantly lower amounts than Set 2. Chemical transformation, identified as a less possible pathway, facilitates the conversion of malathion to malaoxon through oxidative degradation [2,22,23].

This comprehensive study on the North Oconee River highlights the complex interplay of diverse chemical compounds, with particular emphasis on pesticides like malathion, and its implications for both environmental and public health. The identification of the 33 compounds, including malathion and other organic compounds, underscores the potential contamination originating from agricultural and urban runoff in the watershed. The consistent presence of these compounds in the river water raises considerable concerns regarding their persistence, bioavailability, and potential long-term effects on the overall biodiversity of the water body. From an environmental health perspective, the elevated concentrations of organophosphates, organochlorides, and PAHs in the North Oconee River suggest an ongoing environmental stressor. The persistence of these compounds throughout the study duration implies a continuous input, possibly from ongoing agricultural and urban activities.

The detection of malathion, a widely used pesticide, in significant abundance prompts serious environmental health considerations. The compound's propensity to accumulate and its persistence in the environment raise concerns about its long-term impact on aquatic ecosystems, potentially disrupting the balance of the flora and fauna within the river. From a public health standpoint, the presence of malathion in the river water raises red flags due to its documented adverse effects on human health. The inhibitory effects of malathion on acetylcholinesterase, as well as its potential association with genetic and chromosomal disorders, highlight the need for careful consideration of its impact on local communities [1,24]. Moreover, the observed increase in malathion concentrations during precipitation events, particularly in December and January, accentuates the potential for pesticide transport into water bodies through stormwater runoff. This phenomenon has direct implications for environmental health, as it may lead to heightened exposure levels for individuals relying on water from the North Oconee River for various purposes, such as drinking or recreational activities. The laboratory experiments examining malathion degradation reveal the significant role of microbial activity in river water. This finding not only contributes to our understanding of the fate of pesticides in natural environments but also underscores the importance of considering microbial degradation of pesticides in environmental risk assessments. The detection of malaoxon, an oxidative product of malathion, raises concerns due to its persistence, increased bioavailability, and enhanced toxicity.

The ongoing study, characterized by extensive spatial and temporal sample collection and analysis, aims to determine if the North Oconee River poses a significant environmental risk. Additionally, present research is actively investigating the specific indigenous microbial degradation pathway responsible for the transformation of malathion into malaoxon and other metabolites. As the research progresses, a comprehensive understanding of the environmental implications and potential risks associated with the presence of malaoxon in the North Oconee River will be achieved.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/environments11050089/s1, Table S1. Experiment conditions for the microbial degradation of pesticides; Figure S1. Schematic flow chart for the extraction of water sample using solid phase extraction; Figure S2. Mass spectra of all compounds identified with high confidence using non-targeted data analysis and molecular ion matches with corresponding spectra in the NIST library.

Author Contributions: Conceptualization, S.D. and A.D.; Formal analysis, S.D.; Funding acquisition, A.D.; Investigation, G.B.; Methodology, G.B. and S.D.; Project Administration, A.D.; Resources, A.D.; Software, S.D.; Supervision, A.D.; Writing—Original Draft, G.B. and S.D.; Writing—Review and Editing, A.D. All authors have read and agreed to the published version of the manuscript.

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