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Fate, Transport, and Impacts of Glyphosate, Glufosinate and Aminomethylphosphonic acid in the Ecosystem and Built Environment

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Civil and Environmental Engineering

by

Pedro Jesse Martin

Dissertation Committee:
Assistant Professor Shakira Hobbs, Chair
Assistant Professor Christopher Olivares
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2024

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DEDICATION

I dedicate this dissertation to my beloved family, whose unwavering support, love, and encouragement have been my guiding light throughout this academic odyssey.

To My Family,

Your enduring companionship and sage advice have fortified me through every challenge, reminding me of the profound significance of this journey. My achievement of a PhD stands not only as a testament to my academic pursuits but also as evidence of the boundless love, support, and dedication that have shaped my path of personal growth. With heartfelt gratitude, I honor their invaluable contributions to my success.

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PUBLICATIONS

Martin, P. J., He, K., Blaney, L., & Hobbs, S. R. (2023). Advanced Liquid Chromatography with Tandem Mass Spectrometry Method for Quantifying Glyphosate, Glufosinate, and Aminomethylphosphonic Acid Using Pre-Column Derivatization. *ACS ES&T Water*, *3*(8), 2407–2414. https://doi.org/10.1021/acsestwater.3c00094

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ABSTRACT OF THE DISSERTATION

Fate, Transport, and Impacts of Glyphosate, Glufosinate and Aminomethylphosphonic acid in the Ecosystem and Built Environment

by

Pedro Jesse Martin

Doctor of Philosophy in Civil and Environmental Engineering

University of California, Irvine, 2024

Assistant Professor Shakira Hobbs, Chair

The prevalent utilization of glyphosate-based herbicides in modern landscaping and agricultural practices necessitates a methodical exploration into the monitoring, forecasting, and assessment of glyphosate's ubiquity and its consequential impacts. The goal of this research was to develop tools for better understanding of the fate, transport, and public health implications of glyphosate, glufosinate and aminomethylphosphonic acid (AMPA) in environmental matrices. The work resulted in the development of robust analytical detection tools for the monitoring of glyphosate-based herbicides in tap, surface, and groundwater sources. Developed method overcame analytic limitations by utilizing 9-fluorenylmethylchloroformate (FMOC-Cl) pre-column derivatization and solid phase extraction (SPE) via liquid chromatography with tandem mass spectrometry (LC-MS/MS) to achieve detection limits lower than World Health Organization (WHO) standards. The spatiotemporal transport and distribution of the target compounds and the vulnerability of various watersheds were investigated via remote sensing (RS) and groundwater interconnectivity models. The trends identified showed varying levels of watershed

pesticide vulnerability across the study areas in Kentucky, USA, and Belize, indicating shifts in sensitivity of the model to environmental and public health influences, with emphasis on karst and alluvial geological settings as higher-risk areas, underscoring the importance of adopting responsible pesticide application methods for achieving sustainable agricultural practices and water source protection. Results from the prediction models and experimental field monitoring data were analyzed and correlated with socio-economic and environmental justice factors to establish a comprehensive understanding of the intricate interplay between agricultural practices, environmental impact, and societal equity. The study reveals a significant portion of groundwater vulnerability to glyphosate contamination is concentrated in densely populated regions due to reliance on aquifer reserves for drinking water. There also exist a disproportionate impact on low-income and Black, Indigenous, and People of Color (BIPOC) communities highlighting the need for equitable planning and sustainable development practices to address environmental disparities and ensure community well-being.

INTRODUCTION

Motivation/Rationale

Many significant environmental laws in the United States and worldwide, aimed at safeguarding water resources from pollution, are consistently undermined by shortcomings in enforcement and monitoring [1]. These deficiencies have left numerous communities susceptible to the repercussions of excessive dependence on glyphosate and glufosinate for agricultural productivity and landscaping [2]. Glyphosate and glufosinate are surge in agricultural applications are attributed to their post-emergent, systemic, and non-selective action along with the development of resistant crops [3, 4]. Together with aminomethylphosphonic acid (AMPA), a principal breakdown component of glyphosate, enters water bodies through runoff and exhibits greater mobility and persistence in aquatic ecosystems [1]. Agricultural baskets in Belize and the Midwest of United States have exhibited expansion of industrial-scale glyphosate-based herbicides and the adoption of glyphosate-resistant crop varieties in boosting crop yields and ensuring food security [5]. Whilst these gains contribute to the attainment of Sustainable Development Goals (SDGs) related to food security and world hunger (SDG 2), the ability to meet environmental sustainability (SDG 15) and clean water access (SDG 6) still lingers [6, 7]. The long-term viability of agricultural systems is undermined by the far-reaching range of interconnected consequences for both human and environmental well-being [7]. This necessitates the urgent need for comprehensive monitoring and regulatory measures to address glyphosate-based herbicides related pollution and protect vulnerable communities from the adverse effects of agricultural chemical use.

However, the controversy surrounding regulations on glyphosate, glufosinate and AMPA inhibits cohesive monitoring, enforcement, and development of integrative plans for the best management plans (BMPs) for the usage of these herbicides [1]. Glyphosate-based herbicides came under intense public scrutiny due to the International Agency for Research on Cancer (IARC), a specialized agency of the World Health Organization (WHO) classifying the compound as a "probably carcinogenic to humans" in 2015 [8]. This view has not been upheld by the Environmental Protection Agency (EPA), asserting that there is no convincing evidence for such a conclusion and hence set a higher maximum contaminant level (MCLs) of 700ppb (compared to the 0.1ppb of IARC) [9]. While the controversy surrounding glyphosate regulation continues and reflects complex scientific, regulatory, and socioeconomic dynamics, it is imperative that proactive measures be put in place to find tools and protocols to address the concerns raised and safeguard public health and the environment. This research aimed to develop analytical tools to measure the amount of glyphosate, glufosinate and AMPA in agroecosystems and comprehend the routes by which it is transmitted. Additionally, an environmental vulnerability assessment of potential glyphosate exports was created, as well as a simulation of the overall impact of physical and biogeochemical processes on water flow and reservoirs. For the purpose of quantifying possible environmental, health, and economic effects of applying glyphosate, the watershed vulnerability assessment was built utilizing inputs such as application rates, transport in soil, water, landforms, and watershed flow rates. Study areas include the watersheds of the Belize River in Belize, and Camden Creek and Cane Run in Kentucky.

Due to glyphosate-based herbicides' ionic nature, high polarity and solubility in water, problematic evaporation, poor solubility in common organic solvents, low volatility, low mass, and preferred complexing tendency, detecting glyphosate residue using straightforward analytical techniques is difficult [10-12]. Furthermore, the absence of chromophore or fluorophore groups in the structure of glyphosate prevents the analytical identification of these herbicides, necessitating pre-column or post-column derivatization [13]. Detecting the presence of glyphosate is difficult without pre-treatment due to glyphosate's ability to adsorb strongly on clay minerals and organic or mineral particles in water, and its high affinity for metal cations that form complexes with it [14]. Glyphosate detection has improved due to advancements in analytical technologies, such as liquid chromatography coupled with mass spectrometry (LC-MS/MS), which offers sensitivity and specificity for detecting glyphosate residues at trace levels [10, 11, 15]. However, solid-phase extraction (SPE) techniques can enhance glyphosate analysis by concentrating and purifying glyphosate and its metabolites from complex samples [16, 17]. This work aimed to develop SPE coupled LC-MS/MS protocols to target glyphosate while minimizing matrix interference, enhancing our understanding of glyphosate fate and environmental impact.

The discharge and movement of glyphosate, glufosinate and AMPA into surface waterways and groundwater conduits can be expedited heavy rains. This transport process may further extend through various layers of rock and soil, ultimately reaching aquifers, which serve as critical subsurface water sources [18, 19]. Although surface waters can sometimes contribute to man-made reservoirs to enable settling time for degradation of these pesticides, aquifers often stand as the primary source of drinking water [10]. Recent

reports indicate that trace amounts of glyphosate and AMPA are in underground water [11]. This presents a critical interest in water quality protection, mainly since many agricultural lands in Kentucky and Belize are located in karst areas [18, 19]. The nature of the groundwater flow system causes these areas to be highly vulnerable to groundwater contamination [20, 21]. Due to the formation of channels and sinkholes, groundwater contamination is a significant problem in karst geology. The inadequately treated runoff, stormwater and wastewater movement into the aquifer is directly contaminated by the pesticides using these channels and sinkholes [10]. The complex hydrological pathways make glyphosate fate challenging to ascertain. Although there are no specific nutrient or pesticide models, glyphosate transformation occurs due to the presence of groundwater, necessitating the development of a numerical hydrologic model. Graphical techniques for allocating pesticide loads to fast, intermediate, and slow flow pathways are robust; however, they have limitations as they do not explicitly quantify the fate and transport trends of glyphosate [22]. Spatiotemporal studies of pesticide pollution via geographic information systems (GIS) and remote sensing (RS) has led to new directions in the study of water resources [23]. Identification of groundwater contamination by pesticide drift and transport using RS data provides indirect analysis of observable terrain features like geology, geomorphology, and their hydrologic characteristics [24-26]. The use of GS and RS platforms will establish a framework for predictive contaminant modeling, addressing the issue of unreliable access to data for pollution characterization [27]. Astmann et al. highlight the impact of physical and chemical processes on pesticide occurrences and drift regulation

characteristics in predictive modeling, particularly in the context of climate change-induced pesticide loads [3].

Water pollution resulting from these climate change-related incidents not only impedes economic development but also poses risks to public health and undermines environmental integrity [28]. Addressing water pollution concerns would improve communities' health, environment, and economics [4, 29]. The pursuit of environmental justice by marginalized communities and advocacy organizations has sparked widespread grassroots activism in response to numerous cases of pesticide drifts and runoffs. As a result, the dangers and benefits of glyphosate herbicides can no longer be evaluated in isolation; they must be compared against alternative measures that sustainably use herbicides. Current environmental assessments in pesticide product development are generally restricted to plant uptake and emissions of active ingredients [4]. The lack of a comprehensive evaluation of the impacts of pesticide manufacturing and application undermines environmental objectives in managing end-of-life processes. The necessity for a comprehensive examination of pesticide effects has become increasingly apparent.

Research Objectives

The dissertation aimed to tackle the multifaceted challenges posed by the widespread use of glyphosate-based herbicides by constructing a comprehensive understanding of glyphosate's prevalence and its consequential impacts on both environmental and public health domains. Utilizing remote sensing technologies and groundwater interconnectivity models, the dissertation aimed to unravel the intricate spatiotemporal dynamics governing

the transport and distribution of glyphosate, glufosinate, and AMPA. The research aimed to provide actionable insights by leveraging a multi-method approach encompassing field experimentation, predictive modeling, and socio-economic analysis for optimal management strategies tailored to diverse rural and urban landscapes. Achievement of the goals describe above were guided by three research question:

RQ1. How do novel detection techniques and analytical tools contribute to enhancing our ability to monitor glyphosate residues in various water sources, and what are the implications of these findings for environmental and public health management?

RQ2. What are the spatiotemporal dynamics governing the transport and distribution of glyphosate, glufosinate, and AMPA within watersheds, and how can remote sensing technologies and groundwater interconnectivity models inform strategies for mitigating environmental impacts?

RQ3. What socio-economic and environmental justice factors influence the interplay between agricultural practices, glyphosate usage, and societal equity considerations, and how can this understanding inform the development of sustainable management practices tailored to diverse rural and urban landscapes?

Intellectual Merit

Despite the persistent debates regarding glyphosate's potential health and ecological consequences, there remains a notable absence of effective adaptive management solutions

tailored to mitigate its impact. Glyphosate's widespread application poses a significant threat to water sources, contributing to contamination and amplifying concerns about pesticide runoff and its detrimental effects. This work addresses critical knowledge gaps surrounding glyphosate usage and its environmental implications, glyphosate detection, fate, and its impact on groundwater quality. The lack of comprehensive methodologies for monitoring and forecasting the presence of glyphosate-based herbicides in various watersheds, both agricultural and urban, highlights the urgent need for innovative approaches. This study took an interdisciplinary approach to explore the complex aspects of glyphosate usage, including its analytical, environmental, and societal dimensions. The research developed new analytical protocols and geospatial modeling methods to monitor, assess, and predict glyphosate levels in various environmental contexts. The detection and measurement of glyphosate, AMPA and glufosinate through the utilization of sample pre-treatment and pre-concentration techniques, in conjunction with LC-MS/MS was utilized. Derivatization pre-treatment was used to enhance sensitivity, selectivity, and accuracy through the modification of the functional groups responsible for stability of analytes during storage and/or analysis, reducing the risk of degradation. SPE minimized matrix interferences and enhanced specific chemical reactions for analyte recovery. Anthropogenic influences in runoff contamination and watershed vulnerability were evaluated through characterizing, modeling, and mapping of site-specific contributors. Statistical correlation of the monitoring data and mapped watershed vulnerability exposed the socio-economic disparities. By addressing these critical knowledge deficiencies, the research aimed to contribute significantly to our understanding of glyphosate's environmental fate and its implications

for ecosystem health. This comprehensive approach informs decision-making processes and facilitates the development of more effective management strategies to mitigate glyphosate-related environmental risks.

Broader Impacts

This work leveraged the expertise of various stakeholders for broader impact across various issue areas, fostering new partnerships and educational opportunities for undergraduate research, teaching, and information sharing to address the goals more effectively. Collaboration with key stakeholders was facilitated by funding from the National Science Foundation (NSF) and National Institute of Health's (NIH) University of Kentucky (UK) CARES program. Analytical method development training at Agilent and the University of Maryland Baltimore County was supervised by Dr. Lee Blaney and Dr. Ke He. UK Cooperative Extension Service Program farms and the Belize Department of Environment (DOE) have been a resource in identifying study areas and avenues for community interactions. Several undergraduates and a high school student have assisted in various roles throughout the execution of the goals of this dissertation. Three undergraduate students are co-authors of published or papers under review. They have presented works at a number of nationally and regionally recognized. The engagement of these students has sparked and nurtured interest in engineering, facilitating the successful transition of two students— one a high school student and the other an undergraduate— into their respective programs, one at the doctoral level and the other at the undergraduate level. A collaboration with a non-profit organization, BioGals, has provided avenues for underrepresented women of color in STEM fields to engage in outreach and field work.

Organization of the Thesis

This dissertation is structured around manuscripts that encompass publications already available, those currently under review, and those in preparation for submission to journals. Consequently, each chapter is designed as an independent paper, complete with its own abstract. Each manuscript endeavors to address the research questions outlined in Table 1.1. The subsequent portion of Chapter 1 delineates the Intellectual Merit and Broader Impacts of the dissertation, delving into scholarly discourse by examining prior research and identifying existing knowledge gaps.

Table 1. 1 Summary of Dissertation Organization

Chapters	Manuscript	Research Questions		
		RQ1	RQ2	RQ3
2	Advanced Liquid Chromatography with Tandem Mass Spectrometry Method for Quantifying Glyphosate, Glufosinate, and Aminomethylphosphonic Acid Using Pre-Column Derivatization	✓		
3	Improved Simultaneous Quantification of Glyphosate-based Herbicides in Urban Watersheds in California	✓		
4	Exploring Pesticide Transport, Groundwater, and Environmental Justice in a Changing Climate: A Community Engaged Research Approach	✓	✓	✓
5	Sustainable Environmental Management via Spatial Modeling and Mapping of Glyphosate Vulnerability Zones in Belizean Watersheds		✓	✓

Literature Review

Chemistry, Usage, and Environmental Impact

Glyphosate, introduced by Monsanto, USA, in 1971, and glufosinate, introduced by AgrEvo, Germany, in 1984, are well-known for their non-selective mode of action, effectively inhibiting the growth of all plants they come into contact with [30]. Various formulation of glyphosate and glufosinate have been the main ingredients in many herbicides used in agricultural and landscape management practices in the United States since its introduction in the 1970s [4]. Glyphosate and glufosinate are organophosphate molecules that contain phosphate ($–PO_3H_2$), carboxylic (–COOH), and amino ($–NH_2$) as functional groups. These phosphorus-containing amino acid-type post-emergence herbicides find extensive use in diverse applications, including weed control in aquatic environments and vegetation management [31]. Glyphosate has a phosphonomethyl glycine backbone, forming a phosphonic acid group, while glufosinate has a phosphinic acid backbone with an amino group. AMPA, the degradation product of glyphosate, shares a similar structure but lacks a carboxylic acid group. These compounds contain various functional groups, with glyphosate featuring a phosphonic acid group, carboxylic acid group, and an amine group; glufosinate containing a phosphinic acid group and an amino group; and AMPA having a phosphonic acid group and an amine group [22, 29, 32]. These functional groups significantly affect their acidity, solubility, and interactions with other molecules.

The evolution of glyphosate usage from exploring its initial development to its current widespread adoption status is attributed to the emergence of resistant crop species [31]. These broad-spectrum herbicides were intended for convenient and effective solution

for weed control in various crops, including soybeans, corn, cotton, and wheat [6, 11]. The genetically modified plants and seeds are resistant to the interference with the enzyme (5-enol-pyruvyl-shikimate-3-phosphate synthase) responsible for catalysis in the shikimic acid pathway [31]. This mode of mechanism by glyphosate and glufosinate inhibits the photosynthesis and respiration of the plant. Between 1995 and 2014, glyphosate application in the U.S. skyrocketed from 28 million to 280 million pounds annually, driven by the introduction of Roundup ready genetically engineered crops, resulting in more frequent and concentrated herbicide use alongside additional herbicides [33]. This revolutionized glyphosate-based agriculture productivity was achieved by minimizing labor costs, allowing for non-selective weed control and application of glyphosate and glufosinate as either a pre-harvest desiccant or defoliant [34].

Approximately, 19 billion pounds were applied globally from 1974 to 2016, making it the most prevalent herbicide worldwide with about 3.6 billion pounds of glyphosate were applied in the US alone [34, 35]. The International Agency for Research on Cancer (IARC) of the World Health Organization and the Environmental Protection Agency (EPA) conducted extensive reviews of glyphosate's safety and potential for cancer in the 2010s. The IARC classified glyphosate as "probably carcinogenic to humans" in 2015, but the EPA has consistently maintained that the chemical is safe when used as directed [9]. These conflicting assessments sparked public debate and intensified legal battles between glyphosate manufacturers and plaintiffs claiming that exposure to the chemical caused cancer diagnoses. As a result, the regulatory status of glyphosate has remained a contentious issue, with calls for stricter laws or outright bans in some jurisdictions. Glyphosate poses various

risks to aquatic ecosystems, including both acute and chronic effects on aquatic organisms and ecosystem functioning [36]. Glyphosate exposure poses significant risks, including toxicity to aquatic organisms, disruption of ecosystem processes, and harmful algal blooms [37]. Minimizing glyphosate contamination, protecting aquatic habitats, and promoting sustainable weed management practices are crucial for preserving aquatic ecosystem health and integrity [1]. The need for a comprehensive understanding of the health impacts of glyphosate, including its potential environmental contamination, water quality, and indirect exposures, is crucial for informed risk assessments and regulatory decisions [6, 38, 39].

The glyphosate dispute is still continuing strong in the 2020s, due to continuous legal actions, regulatory evaluations, and scientific studies that are influencing the product's future. The legal cases of Johnson v. Monsanto Company (2018), Pilliod v. Monsanto Company (2019), and Hardeman v. Monsanto Company (2019) have generated substantial discourse regarding the possible health hazards linked to glyphosate exposure, as well as the safety of one of the most extensively used herbicides globally [40]. Key discovery from these trials was the revelation of a collection of 141 confidential company documents used by the manufacturers to manipulate scientific research and public perception to favor their product [40]. This revealed ghostwriting articles, interfering with peer review processes, and creating a biased academic website to discredit independent research highlighting glyphosate's toxicity similar to the strategy used by the tobacco industry [34, 41, 42]. These efforts misled policymakers and the public, potentially compromising human health and the environment by promoting the widespread use of glyphosate in industrial agriculture [41, 42]. The verdicts from these trials not only set precedence for further ligation on the impacts

of glyphosate and glufosinate but continue to fuel the scrutiny and debates on the varied efforts to streamline related regulations [40]. Alternative weed management practices, such as integrated pest management (IPM) strategies and new herbicides and genetically modified crops, have the potential to reduce glyphosate dependence, influence consumer preferences, and ensure regulatory decisions and legal outcomes continue to influence its usage in agriculture and land management practices.

Traditional Monitoring Techniques for Glyphosate, Glufosinate and AMPA Residues

Understanding and predicting the transport of glyphosate, glufosinate, and AMPA necessitates measuring them across diverse concentrations and examining their interactions with water complexity, posing significant technical and financial challenges due to complex molecular interactions and the absence of suitable real-time measurement technology [33]. Achieving simple, portable, and cost-effective measurement methods remains a critical yet elusive goal, particularly given the wide range of environmental conditions [43]. However, huge efforts have been made in the development of analytical detection methods despite these challenges. Traditional analytical methods such as chromatography, mass spectroscopy, electrochemical sensors and spectroscopic methods have been used to break down matrices for effective separation and analysis. The various spectroscopic methods in the development of protocols for measuring glyphosate, glufosinate, and AMPA relies on the wavelength frequency between the compounds and electromagnetic radiation. The earliest application of this principle via nuclear magnetic response (NMR) was presented by Dickson et. al in 1998 [44]. However, this application was limited by the high detection limit of 1

mg/mL and the longer sample preparation and run times. Cartigny et. al [45] attributes these challenges in applying NMR for detecting organophosphates such as glyphosate, glufosinate, and AMPA to the false positives generated by the overlapping of similar signals. A conclusion was therefore drawn that NMR could be utilized for rapid qualitative confirmation of the presence of the compounds. Significant sensitive can be achieved with Surface-enhanced Raman scattering (SERS) using gold nanorods as substrates, however, its applications is limited until the further research to understand complex interactions with organic compounds and metal ions [46]. Zhao et. al [47] and Homola et. al [48] proposed protocols via chemiluminescence-molecular imprinting sensor (CL-MIS) and surface plasmon resonance (SPR) respectively. While these methods offer rapid analytical capabilities for glyphosate detection, they are not be as effective for identifying its primary metabolite, AMPA [47, 48].

Several applications for analyzing glyphosate, glufosinate, and AMPA in various matrices have used capillary electrophoresis (CE) to highlight the versatility of this technique. Chang et al. [49] coupled CE with indirect fluorescence detection employing fluorescein and an argon-ion laser to separate analytes within 5 minutes although application in groundwater proved problematic. Corbera et al. [50] improved sensitivity for glyphosate and AMPA analysis in natural water by pre-concentration to yield recoveries of 84% for glyphosate and 85% for AMPA, with detection limits of 85 μg/l for glyphosate and 60 μg/l for AMPA. These reported limits are still higher than IARC maximum contaminant limits. The proposed method by Cikalo et al. [51] reported detection limits were 0.8 μg/ml for glyphosate with linear calibration ranges of 1.7-170 μg/ml for glyphosate and 1.1-110

μg/ml for AMPA due to the lack of significant ultraviolet (UV) absorbance for direct injection analysis. Similarly, Peruzzo et al.'s [52] UV detection with high performance liquid chromatography method for glyphosate in surface waters after pre-column derivatization with FMOC-Cl resulted in a detection limit of 0.7 mg/L. Another common detection method for the analysis of glyphosate and AMPA in water is ion chromatography (IC). The earliest work with IC is exhibited by You et al. [53], where condensation nucleation light scattering detection (IC-CNLSD) for direct analysis of glyphosate and AMPA in water resulted in the achievement of detection limits of 41 μg/l for glyphosate and 53 μg/l for AMPA. However, this cation-exchange column application could not detect none of the analytes in environmental matrices. Bauer et al. [54] implemented IC with electrospray mass spectrometry detection for glyphosate, AMPA, and other polar organic pollutants in water but rather opted for an anion-exchange column with gradient elution and achieved concentration levels of 1 μg/l in ground and surface water without additional sample preparation.

Applications for glyphosate, glufosinate and AMPA via gas chromatography (GC) are very limited due to the chemical properties of the compounds renders them thermally unstable requiring extensive derivatization to overcome their polar nature and high-water solubility. Detection limits of 8, 12, and 20 μg/l for AMPA, glyphosate, and glufosinate, respectively, with high recoveries were achieved by Kataoka et al. [55] using flame photometric detection. Borjesson et al. [56] developed a coupled pretreatment method using acidification and derivatization with trifluoroacetic anhydride (TFAA) and trifluoroethanol

to achieve similar results. The intense labor and analytical deficiencies for GC makes it challenging for monitoring concentrations of glyphosate, glufosinate and AMPA.

Advances in Mass Spectroscopy Measurements

Recent advancement in mass spectrometry technology has led to the development of several protocols via LC-MS/MS due to its higher sensitivity [30, 57-61]. LC-MS/MS applications offer significant reproducibility and recovery for glyphosate, glufosinate and AMPA protocols due to the presence of electrospray ionization as an ion source. The LC-MS/MS technology essentially provides noise reduction capabilities and ensures reduction in false positives [62]. Although LC-MS/MS is a cost intensive option for detecting glyphosate-based herbicides, it minimizes the challenges related to column life, sample preparation, sample preservation and interactions with metal ions. Laborious time-consuming protocols are eliminated by the adoption of LC-MS/MS technology for the analytical detection of organophosphate [12]. The sensitivity of the LC-MS/MS, which combines two mass analyzers for the first stage filtration of precursor ion before its fragmentation with high energy, and the second stage filtering of produced ions generated by fragmentation, is enhanced further with derivatization of glyphosate, glufosinate and AMPA [57, 61].

Either pre-column or post-column, derivatization provides these compounds with the chemical chromophore and fluorescence groups that enhance detection by conventional technology [11, 63-65]. Most pre-column techniques rely on derivatization with 9-fluorenyl-methyl chloroformate to produce fluorescent derivatives and reduce the analytes' polarity

to aid chromatographic retention while post-column reaction largely depends on o-phthalaldehyde with either N, N-dimethyl-2-mercaptoethylamine, or mercaptoethanol [63, 66-71]. 9-fluorenyl-methyl chloroformate (FMOC-Cl) derivatization has been proven to be simple and successful. Effective FMOC-Cl derivatization is guaranteed with the addition of sodium tetraborate buffer (borate) and ethylenediaminetetraacetic acid (EDTA) to ensure complete derivatization and prevents the metallic complexation of the glyphosate during analysis [12, 69]. Recent advancements in analytical techniques have led to the development of efficient methodologies combining LC-MS/MS with preconcentration techniques for detecting derivatized glyphosate, glufosinate, and AMPA in water, addressing the need for heightened sensitivity and selectivity, with negative ionization aiding in molecular fragmentation of these highly polar and volatile compounds [63].

Utilization of Preconcentration Sorbents

Employing solid-phase extraction (SPE) enhances the efficiency of conventional detection methods for quantifying polar compounds in water samples [43]. Solid-phase extraction (SPE) separates analytes based on their hydrophilic-lipophilic retention capacities, especially significant for glyphosate-based target analytes owing to their multiple pKa states; hence, reverse-phase SPE is favored over normal-phase SPE to avoid sorbent drying concerns [17]. This is accomplished by conditioning the sorbent with organic solvents, enhancing analyte retention during reverse-phase SPE. Ibanez et al. [63] showed recoveries were ranging from 89 to 106% for glyphosate (RSD < 9%), from 97 to 116% for AMPA (RSD < 10%), and from 72 to 88% for glufosinate (RSD < 12%) when LC-MS/MS was

utilized in conjunction with preconcentration of derivatized analytes via Oasis HLB SPE cartridges. Ghanem et al. [58] devised an LC-MS method involving extraction with sodium hydroxide, purification on an anion-exchange resin, and derivatization with FMOC-Cl on the same Oasis HLB SPE cartridge and achieved LODs of 20 and 30 μg/l for glyphosate and AMPA, respectively.

Spatiotemporal Dynamics of Glyphosate Transport and Distribution

The transport of glyphosate, glufosinate and AMPA throughout watersheds is largely impacted by several variables such as climate, soils, hydrology, geomorphology, land use/land cover (LULC) and anthropogenic factors [25]. Both overlay methods and statistical inference-based models do have shortfalls leading to assumptions and errors. However, modified overlay models perform better and provide the closest realistic simulations of the analytes with watersheds [72]. The most common overlay method utilized for watershed vulnerability assessment is DRASTIC [73]. Recent findings from modified DRASTIC have tailored field investigations and regional characteristics to yield the best results, especially in arid, semi-arid, basaltic, agricultural, and landfill areas. Panagopoulos et al. [74] improved the DRASTIC model by adjusting the weightings and ratings of its parameters using statistical and geostatistical methods, leading to the replacement of the hydraulic conductivity thematic layer with LULC which led to significantly enhanced the correlation of the groundwater vulnerability index and analyte concentrations by 33%.

However, Leone et al. [75] noted that the interpolation of limited field data introduces errors contributing to the imbalance in weighing thematic factors. The introduction of analytical hierarchy process in overlay methods significantly improves the efficiency and robustness of this approach. The adoption of AHP inherently minimizes the inconsistencies with the model's parameters and ensures comparative assessment of the influencing factors [25]. A combined watershed vulnerability assessment with GIS spatial analysis tools to map susceptible zones showed significant vulnerability to contamination in agricultural areas and significant correlations between water quality parameters and vulnerability, suggesting the method's potential applicability in other watersheds for accurate susceptibility assessments [76]. Rahaman et. al [77] underscore the importance of varying relative matrix comparison due to differences in regional characters and influences of anthropogenic characteristics. The normalization of these qualitative descriptors have been translated effectively using the Saaty's scale [78]. The Saaty Scale in AHP-based watershed vulnerability assessments provides a structured approach for quantifying qualitative judgments based on stakeholder input to determine the relative importance of factors. This evaluation and prioritization enhances decision-making and improves the reliability of the assessment process [78].

Socio-Economic and Environmental Justice Considerations

The efforts to address the disparity in environmental impacts of glyphosate-based herbicides and safeguard human and public health must align with systemic causes of environmental problems and ensuring fair participation in environmental decision-making

[79]. Through this approach, fair treatment and meaningful involvement of all stakeholders will ensure no disproportionate bearing of the environmental burdens due to disempowerment. The widespread use of glyphosate-based herbicides is linked to various adverse environmental and health effects, including flooding, extreme weather, food insecurity, and chronic diet-related diseases [80]. The over-reliance has led to lackluster approach to industrial agriculture as exhibited in the 2019 heavy floods of the midwestern states in the U.S., which led to an estimated loss of billions of dollars and contributing to rising food prices and decreased food supply [34]. Industrial farms no longer invest time and resources in proper soil management and health for catchment of these runoffs due to the non-selective mechanism of glyphosate-based herbicides [81, 82]. The burden cause by the disproportionate usage of glyphosate-based herbicides has the potential of socio-economically depriving low-income and Black, Indigenous, and People of Color (BIPOC) communities of their quality of life. Cushing et. al. [83] revealed that areas with high percentages of BIPOC and predominantly low-income families of Hispanic descent in California experienced significant disparities in exposure to hazardous pesticides. A critical trend between the quality of life for children in several California counties and the occurrence of organophosphate was also highlighted by Eskenazi et. al [84]. The high use of pesticides in inner-city housing by city authorities and contracted companies leads to increased exposure for residents, who are predominantly BIPOC and low-income families [2].

CHAPTER 2

ADVANCED LIQUID CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY METHOD FOR QUANTIFYING GLYPHOSATE, GLUFOSINATE, AND AMINOMETHYLPHOSPHONIC ACID USING PRE-COLUMN DERIVATIZATION

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This chapter addresses research question 1.) How do novel detection techniques and analytical tools contribute to enhancing our ability to monitor glyphosate residues in various water sources, and what are the implications of these findings for environmental and public health management? The chapter underlines the development and optimization of a robust analytical detection protocol for glyphosate, glufosinate, and AMPA.

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Article

Advanced Liquid Chromatography with Tandem Mass Spectrometry Method for Quantifying Glyphosate, Glufosinate, and Aminomethylphosphonic Acid Using Pre-Column Derivatization

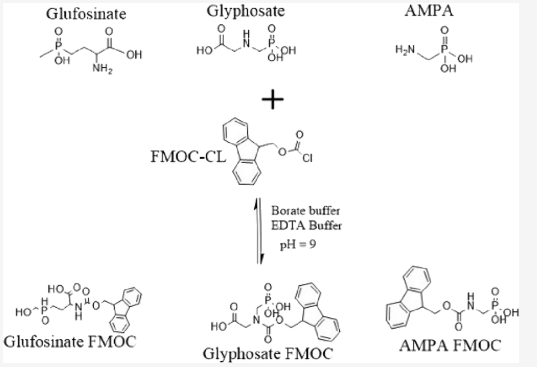
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ABSTRACT: Analytical limitations make it challenging to develop effective methodologies for understanding glyphosate-based herbicide levels in drinking water and groundwater. Due to their lack of chromophores and zwitterionic nature, glyphosate-based herbicides are difficult to detect using traditional methods. This paper offers a straightforward method for quantifying glyphosate, glufosinate, and aminomethylphosphonic acid (AMPA) via 9-fluorenylmethylchloroformate (FMOC-Cl) pre-column derivatization and analysis by liquid chromatography with tandem mass spectrometry (LC−MS/MS). Method development was focused on optimizing the critical variables for optimal derivatization using a 2^4-factorial design. We found that complete derivatization significantly depends on the inclusion of borate buffer to create the alkaline conditions necessary for aminolysis. Ethylenediaminetetraacetic acid (EDTA) addition was critical to minimize metallic chelation and ensure reproducible retention times and peaks. However, EDTA concentrations ≥5% decreased peak intensity due to ion suppression. The FMOC-Cl concentration and derivatization time exhibited a direct proportional relationship, with the complete reaction achieved with 2.5 mM FMOC-Cl after 4 h. Concentrations of FMOC-Cl greater than 2.5 mM led to the formation of oxides, which interfere with the detection sensitivity and selectivity. Desirable results were achieved with 1% EDTA, 5% borate, and 2.5 mM FMOC-Cl, which led to complete derivatization after 4 h.



KEYWORDS: *reversed-phase chromatography, pesticide, micropollutant, glyphosate-based herbicides, pre-column derivatization, glufosinate, aminomethylphosphonic acid (AMPA)*

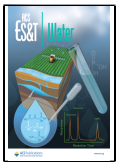
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1. INTRODUCTION

The widespread occurrence of multiple pesticides in rivers and streams due to increased usage creates a complex exposure of compounds potentially toxic to environmental and public health.[1] Glufosinate and glyphosate are broad-spectrum, nonselective, synthetic herbicides introduced in the 1970s for post-emergent weed management in several agricultural and non-crop applications.[2] The use of glyphosate-based herbicides in agricultural operations has increased since the emergence of resistant plants, with an estimated yearly growth rate of 6.8% by 2024.[3,4] The main metabolite, aminomethylphosphonic acid (AMPA), is most likely to be found because glyphosate has a short half-life.[5] This breakdown product is frequently observed in surface waters and is produced by microbial organisms in soil and water.[4,6]

Recent studies indicated that herbicide residues have been discovered in sources of drinking water as a result of widespread and intensive use, which has an increasingly substantial negative influence on the environment,[5−9] leading to the relevance and continued interest in routine monitoring of glyphosate, glufosinate, and AMPA. Several regulatory bodies are increasing the allowable residual limits after evaluating the carcinogenic hazard of glyphosate-based pesticides. In its regulatory investigations, the Environmental Protection Agency found that glyphosate generally has low toxicity to mammals. The World Health Organization nevertheless classified glyphosate as possibly carcinogenic to humans.[4,6,10] As a result, there is controversy on the potential for toxicity and the optimal analytical approach to detect and quantify glyphosate, glufosinate, and AMPA in environmental matrices. The societal upheaval this problem has brought about has raised awareness of the discovery of glyphosate and AMPA in environmental samples. Appropriate methods to assess glyphosate, glufosinate, and AMPA at μg/L levels in aqueous samples are missing.[2,11,12]

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Glyphosate-based compounds are commonly determined using chromatographic methods, such as reversed-phase and cation-exchange chromatography.[13−15] Carboxylic phosphorylated polar herbicides like glyphosate have poor peak shapes and signals in mass spectrometry (MS) detectors due to poor ionization required for effective chromatographic separation. Metallic complexation also contributes to the poor peak shape during chromatography.[16] Traditional technologies, such as gas chromatography (GC) and ion chromatography (IC), have demonstrated limitations in detecting the presence of glyphosate in water.[17] Due to poor analytical reproducibility and sensitivity, glyphosate is difficult to measure due to complicated transitions in GC analysis.

Due to the existence of enantiomeric forms, some GC techniques detect glyphosate, glufosinate, and AMPA as multiple peaks, restricting quantitative measurement.[18] While GC can relatively detect glyphosate and its derivatives in a sensitive and selective manner, the derivatization reaction produces unstable byproducts and is also very time-consuming. Polar organic micropollutants in water samples have been analyzed using ion chromatography with inductively coupled plasma (ICP) mass spectrometry. Although the detection limits were high, glyphosate and AMPA were identified in groundwater and surface water.[19]

New trends and interest in derivatized analyte detection using MS techniques have led to the development of efficient methodologies that combine liquid chromatography (LC) and MS.[20,21] The use of liquid chromatography with tandem mass spectrometry (LC−MS/MS) improves the sensitivity and selectivity of detecting glyphosate, glufosinate, and AMPA in water. Chemical deprotonation allows for herbicide separation and retention on chromatographic columns.[22] However, when employing this quantitative tool, there are numerous causes of uncertainty, including matrix effects, sample loss, physical and chemical interferences, and instrument detector drift. Detection via LC has reached limits of detection (LODs) as low as 20.0 μg/L, but to ensure fast, sensitive, and repeatable analysis of herbicides, derivatization processes and high-end equipment are required.[23]

Derivatization techniques have been employed in a number of studies to identify glyphosate-based molecules.[4] Despite their benefits, these technologies are time-consuming and resource-intensive to operate. Pre- or post-column derivatization provides these compounds with chromophoric or fluorescent groups that enhance detection by conventional analytical instruments.[13,22,24,25] Most pre-column techniques rely on derivatization with 9-fluorenylmethylchloroformate (FMOC-Cl) to produce ionizable derivatives that reduce polarity and aid chromatographic retention. Post-column derivatization shortcomings include the additional post-column dead volume required from large reaction loops, which reduce separation efficiency, and baseline variations caused by additional system noise from multiple delivery lines. Additional downsides include the need to regularly prepare samples for optimal sensitivity or keep them in an inert atmosphere to maintain their reactivity over time.[4,10] For example, after chromatographic separation of the target compounds with a strong cation-exchange column, *o*-phthalaldehyde derivatization was utilized as a post-column derivatization since it is swift, but the derivatives are unstable after a few minutes.

In contrast, pre-column FMOC-Cl derivatization has been proven to be simple and successful.[14,21,22,26−28] However, derivatization with FMOC-Cl is slower than with *o*-phthalaldehyde, and various reaction times have been proposed.[4,10] There is some uncertainty due to the conflicting reports on the importance of reaction times for derivatization. The complete reaction of the glyphosate ion with FMOC-Cl guaranteed stability and successful chromatographic separation on LC columns.[10] The derivatization period had a significant impact on issues related to acidification, buffer concentrations, and the generation of derivatization byproducts, such as FMOC-OH. The chromatographic analysis of glyphosate may be hampered by similar chromatographic transitions as the derivatives.[29,30]

Despite the gains in characterizing glyphosate in drinking water sources, more effort is needed to improve the robustness of detection techniques by eliminating false positives and matrix effects while determining trace levels. The objective of this study was to develop quantitative techniques to identify glyphosate, glufosinate, and AMPA by minimizing peak tailing and increasing retention times with sharper independent peaks to achieve lower detection limits. This research developed pre-column treatment techniques for LC−MS/MS detection of glyphosate-based herbicides at μg/L levels.

2. MATERIALS AND METHODS

2.1. Reagents and Materials. Glyphosate (99%), AMPA (99%), and glufosinate-ammonium (99%) were obtained from Chemservice (West Chester, PA). Primary stock solutions containing 1 μg/μL concentrations for all chemicals (corrected for purity) were prepared in deionized (DI) water. A 100 μg/L working solution of glyphosate, glufosinate, and AMPA was prepared and stored at 4 °C. For sample fortification and calibration standards, several combined solutions of all target compounds ranging from 0.5 to 10 μg/L were created and employed as spike solutions. Isotopically labeled standards, 1,2-$^{13}C_2{}^{15}N$ glyphosate (99%) and $^{13}C^{15}N$ AMPA (99%), were obtained from Cambridge Isotope Laboratories (Andover, MA). The internal standard solution was made in DI water with a 50 μg/L concentration. LC−MS grade water, EDTA, acetonitrile (ACN), sodium tetraborate, FMOC-Cl, methanol (CH_3OH), and phosphoric acid (H_3PO_4) were obtained from Fischer Scientific (Hampton, NH). A 0.1% phosphoric acid (v/v) in ACN/DI water (70:30) was used for rinsing the autosampler before and after injection.

2.2. Analytical Equipment and Conditions. The LC−MS/MS system included an Agilent Series 1290 LC and an Agilent 6470A triple quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA). The LC was equipped with a binary pump, column oven, ultraviolet detector, and autosampler. A Phenomenex Gemini NX-C18 column (3 μm particle size, 100 mm length, and 2.1 mm internal diameter) was used with a 0.4 mL/min solvent flow rate. The column compartment temperature was set to 40 °C, and the injection volume was 20 μL. Mobile phase A was 5 mM ammonium acetate in LC−MS grade water, and B was pure ACN. For the separation, the LC gradient was as follows: isocratic from 0 to 2 min (90% A, 10% B); linear increase of B from 10 to 25% for 3 min; linear increase of B from 25 to 50% for 3 min; linear decrease to 10% B for 1 min; and isocratic for 3 min (90% A, 10% B) to re-equilibrate the column to initial conditions. The method run time was 12 min. Following LC separation, negative mode electrospray ionization was used to introduce the analytes into the MS. The settings included a drying gas flow of 5 L/min, a drying gas temperature of 300 °C, a

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nebulizer pressure of 45 psi, and a 110 V fragmentor voltage. One precursor and one daughter ion were monitored for each compound, along with the retention times and mass/charge ratios. The mass transition ion pairs for the target ions are shown in Table 1.

Table 1. Summary of Multiple Reaction Monitoring Run Conditions, Including the Precursor and Daughter Ions[a]

FMOC-Cl compounds		PI	QI	CE
glufosinate FMOC-Cl	Q	402.1	180	8
	q	402.1	206	16
glyphosate FMOC-Cl	Q	389.9	168	12
	q	389.9	63	66
AMPA FMOC-Cl	Q	332.1	110	4
	q	332.1	136	14
isotope-labeled +4-AMPA FMOC-Cl	Q	336.0	114	4
isotope-labeled +3-glyphosate FMOC-Cl	Q	391.9	170	12

[a]PI, precursor ion; QI, quantitation daughter ion; CE, collision cell energy; Q, quantification transition; q, confirmatory transition.

2.3. Pre-Column Derivatization with FMOC-Cl. A 40 mL aliquot was pipetted into an amber glass bottle along with 800 μL of the internal standard solution, which was added to correct matrix effects. The pH of the matrix and standard solutions was adjusted to 9 by adding 2 mL of borate buffer, 2 mL of EDTA solution, and 6 mL of FMOC-Cl stock solution. The borate buffer ensured proper sample derivatization conditions, whereas the FMOC-Cl agent increased the molecular weight and stability of the analytes of interest for chromatographic separation. Metallic chelation was eliminated with the EDTA addition. Then, samples were placed in a water bath at 40 °C in the dark. To stop the derivatization, 2.4 mL of the phosphoric acid solution was added and kept at 4 °C. For effective chromatographic separation, the effects of the EDTA buffer strength, borate buffer strength, FMOC-Cl concentration, and derivatization time were investigated and optimized, as shown in Table 2.

Table 2. Investigated Factors That Affect the Effectiveness of FMOC-Cl Derivatization Glyphosate, Glufosinate, and AMPA

derivatization factor	varied values
time (h)	0, 1, 2, 4, 8, 24
borate (%w/v)	0, 1, 5, 10
EDTA (%w/v)	0, 1, 5, 10, 20
FMOC-Cl (mM)	1, 2.5, 5, 10, 20

2.4. Design of Experiments for Variable Optimization. A 2^4 factorial design was employed to assess the effects and interactions of FMOC-Cl concentrations, derivatization times, borate buffer concentrations, and EDTA buffer concentrations for best-performing parameters in Table 2 (Figure 1). The best-performing variables from Table 2 were evaluated. JMP statistical software (Cary, NC) was used to build a desirability coefficient to discover which combinations of the different factors resulted in the most desirable outcomes. The four major effects and three two-way interactions were the focus of the desirability function's experimental goal, which was to determine the settings of the variables to optimize the compound responses. The response variable is converted to a 0−1 scale via the desirability function. The reaction scale goes from 0 for the lowest response to 1 for the strongest response. The overall desirability is created by combining each individual response's desirability using the geometric mean to concurrently maximize several responses.

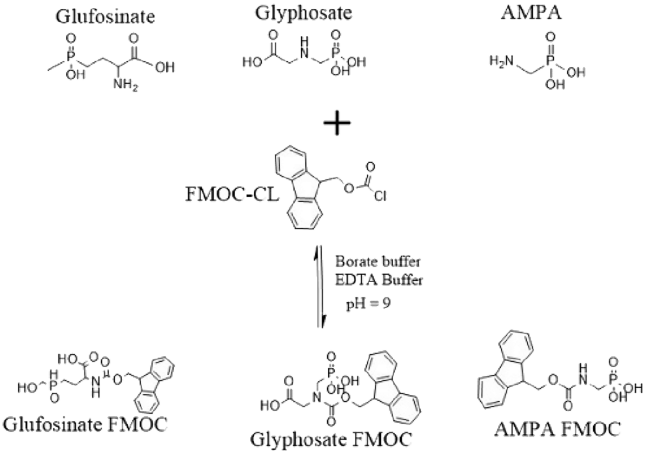


Figure 1. Derivatization reaction between glyphosate, glufosinate, AMPA, and FMOC-Cl.

2.5. Method Validation. The retention times, mass/charge ratios, and relative ion ratios were used to identify the target compounds. The linearity of the method was validated using seven standard solutions in triplicate. The LOD and limit of quantification (LOQ) were determined using various sample and standard spikes ranging from 1 to 10 μg/L in DI water. Spiked duplicates were employed to ensure accuracy and precision. The LOD and LOQ for each analyte were established as the lowest concentration that yielded signal-to-noise ratios (S/N) of 3 and 10, respectively, by gradually reducing the spiked analyte concentrations to achieve the closest values corresponding to those S/N ratios. Method repeatability and interday precision were determined by applying nested design, evaluating three replicates daily at low concentrations (10 μg/L) and three at high concentrations (100 μg/L) on three consecutive days.

3. RESULTS AND DISCUSSION

3.1. Evaluation of FMOC-Cl Derivatization. *3.1.1. Effect of Borate Addition.* Figure 2 highlights the response of the various borate buffer concentrations (w/v) tested to determine the best conditions: 0 (control), 1, 5, and 10%. The reaction did not occur in the control, for which no borate buffer was added. This performance can be attributed to the requirement of alkaline conditions necessary for complete aminolysis shown in Figure 1. When borate buffer was added to the reaction medium, the peak intensities of the derivative products were greater. The reactivity of glyphosate's amino group was enhanced by increasing the buffer concentration, which improved the derivatization reagent's solubility.[31−33] The addition of 1% borate greatly improved the peak response of glyphosate, glufosinate, and AMPA compared to the control (Figure S1). However, when the concentration of the borate buffer was increased to 5 and 10%, a significant compound response was seen ($p < 0.05$). The correlation between the compound response and the reaction medium's alkalinity was established as a result of the rise. However, a significantly higher response was obtained with 5% borate addition compared to the control than the other variables ($p < 0.05$). This observation underlines the requirement for borate to

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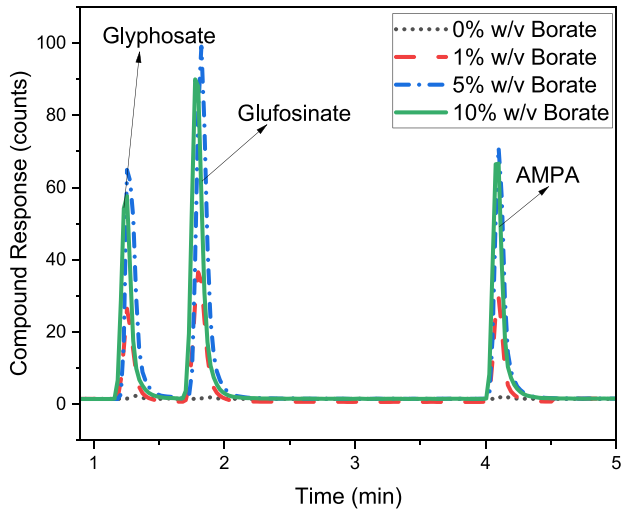


Figure 2. Chromatographic response of target compounds with varied borate concentrations.

ensure full derivatization. The alkaline conditions created by the 5% borate buffer support Ehling and Reddy's results about the optimum pH range of 8−10 as the concentration slightly increases the pH and reduces the compound response.[32]

3.1.2. Effect of EDTA Addition. The performance of the EDTA buffer concentration was evaluated with 100 μg/L (each) standards of glyphosate, glufosinate, and AMPA (Figure 3). The control reaction without EDTA had a higher variation

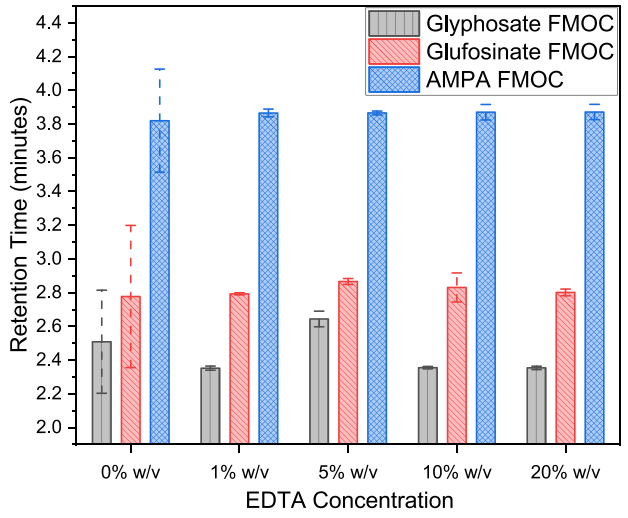


Figure 3. Highlights variation in retention times of target compounds with varied EDTA concentrations (n = 3, error bar = standard deviation).

in analyte retention times (Figure S2). That variation was significantly reduced by introducing 1 and 5% EDTA buffer (p < 0.05). However, EDTA concentrations ≥5% reduced the abundance of all compounds compared to the control and 1% EDTA levels. Except for the 1% EDTA solution, all EDTA levels exhibited an adverse impact on abundance (Figure S3). For all three target compounds, the peak abundance with 1% EDTA was significantly higher than the control (p < 0.05) and 5% EDTA (p < 0.05) conditions. The addition of the EDTA solution minimized the shift in retention times in multiple runs and ensured stable peak abundance results (Figure S2). This trend was true for each of the three chemicals. The EDTA addition eliminated the poor intensity correlated to trace metal contamination in the LC−MS/MS system and enhanced detection of the polar phosphorylated herbicides (Figure 4).

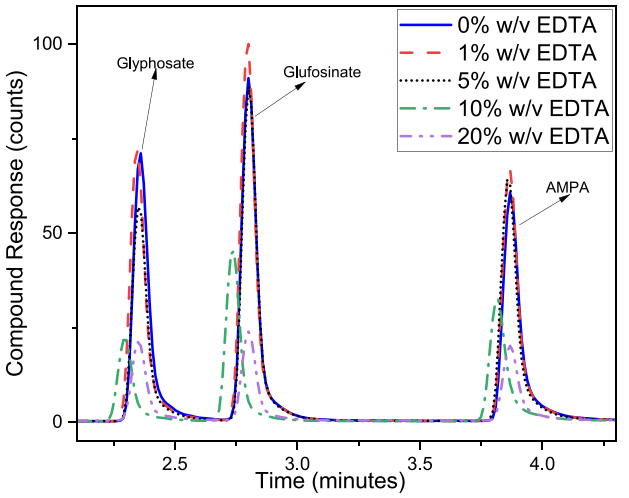


Figure 4. Highlights chromatographic response of target compounds with varied EDTA concentrations.

In LC−MS investigations, it is frequently noted that phosphorylated chemicals and organic acids with numerous carboxylate groups produce poor peak shapes and signals. The phenomenon was also observed for glufosinate, AMPA, and glyphosate.[34] The presence of trace metals, notably iron, contributed from several sources inside the chromatographic system is the reason for the poor peak shape. EDTA was used to address the trace metal contamination problem.[35] EDTA, although a potent metal chelator, caused ion suppression on columns when retained for the higher concentrations >1%.[36] Chelation decreases efficiency and symmetry due to the interaction between the mobile phases, additives, sources other than solute/stationary phase interfaces, and to various degrees depending on the instrument.[34] The compound response in the 1% EDTA solution emphasizes the importance of removing poor peak shapes caused by multiple charged negative-ion analytes.

3.1.3. Effect of FMOC-Cl Concentration. Results demonstrated an improved FMOC-glyphosate area when the concentration of the derivatization reagent was raised from 1.0 to 2.5 mM (Figure 5). However, at the 5 and 10 mM FMOC-Cl concentrations, a negative effect was observed for the FMOC-glyphosate area (Figure S4). FMOC-glyphosate concentrations >2.5 and <20 mM experienced a considerable decrease in the chromatographic response. This adverse performance was attributed to the formation of oxides of the derivatization agent. The interaction between FMOC-Cl and water causes FMOC-OH to be produced at a higher concentration of the derivatization reagent.[11] Because it is poorly soluble in water and has the potential to precipitate, this byproduct can hinder glyphosate detection. As a result, high FMOC-Cl concentrations affect chromatographic separation and reduce FMOC-glyphosate ionization.[30] Even though precipitation was observed with addition of 20 mM FMOC-Cl, all target chemicals responded significantly better (p < 0.05). The excess FMOC-OH can be removed by solid-phase extraction.[8]

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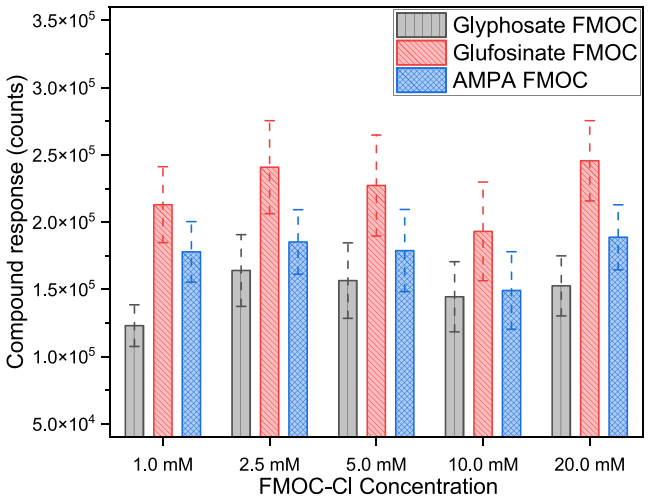


Figure 5. Effect of the FMOC concentration on the chromatographic response (n = 3, error bar = standard deviation).

3.1.4. Effect of Derivatization Time. Figure 6 highlights the investigation into the impact of time on the derivatization process to establish the best time to convert the target molecule into MS ionized products. The length of derivatization ensured sufficient interaction of the analytes with the derivatizing reagent and complete conversion into identifiable ionized compounds. The reaction times required for the reaction of FMOC-Cl by glyphosate have been reported as 2−24 h for complete derivatization.[10] It was however evident that the derivatized products were not stable before 4 h and showed any significant variations in the peak areas.[4] Results demonstrated that the glyphosate, glufosinate, and AMPA derivative products peaked and remained stable after 4 h (Figure 6). Additionally, the derivative products did not exhibit any appreciable fluctuations in response for either, glyphosate, glufosinate, or AMPA. The minor decline in the glyphosate response for derivatization times after 4 h could be attributed to the conversion to AMPA as a relatively proportional increase is exhibited.[10]

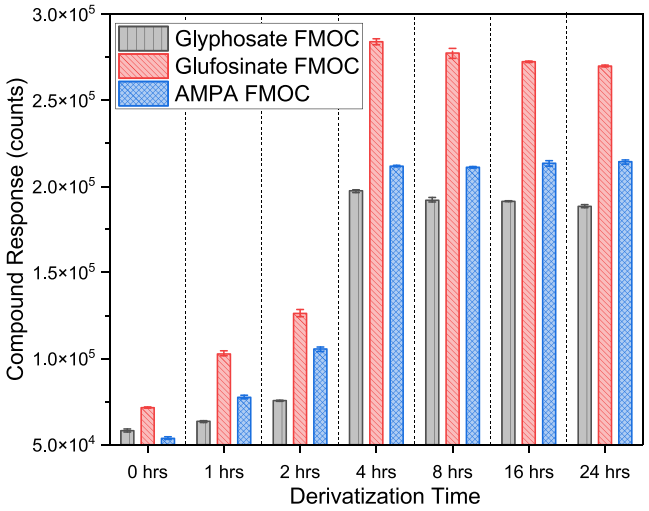


Figure 6. Effect of the time of derivatization on the chromatographic response (n = 3, error bar = standard deviation).

3.2. Optimization of Derivatization Factors. Figure 7 depicts the scenarios regarding the desirability of optimizing the derivatization factors through the interactions of the individual responses. The desirability function accounted for the simultaneous effect optimization for the top two performing alternatives after the individual response investigation. The optimization indicated that the optimal derivatization time for a complete reaction was 4 h, as most combinations of the other derivatization factors investigated performed best under the short derivatization time. All best-performing circumstances also involved the lower concentration of the derivatizing agent FMOC-Cl, whereas the higher concentration resulted in poor performance irrespective of time. The optimal derivatization occurs at 1% EDTA, 5% borate, and 2.5 mM FMOC-Cl after 4 h (Table S1). The interrelationship of the derivatization variables provided insight into the analysis of target chemicals for abundance in trace quantities while reducing background noise (Figure S6). The optimization evaluation improved the water analysis analytical technique by applying compound-specific stable isotopes.[11] Although various variations of derivatization agent and times resulted in weaker performances (Figure 6), the addition of borate was critical to the effectiveness of FMOC-Cl derivatization of glyphosate, glufosinate, and AMPA (Figure 2). The interaction profiles for the various derivatization factors are highlighted in Figure S6. When the concentration of the EDTA was kept at 1%, all target compound responses were higher for the 5% borate addition than for the 10% alternative. However, when the EDTA concentration was 5%, the response for 10% borate was significantly higher than 5% borate ($p < 0.05$). There is an interaction between the concentration of the borate buffer and the time of derivatization, such that for the longer derivatization time, the 5% borate addition had a higher compound response than the 10% borate addition. However, the shorter derivatization time recorded a similar performance for both alternatives of the borate concentrations. This result indicates that a complete reactivity with the target compounds can be achieved in the shortest time possible with either borate concentration. Hence, 5% borate was selected for the optimized method. The interaction between borate and FMOC-Cl concentrations demonstrated that the 10% borate buffer performed better than the 5% borate buffer variable when the concentration of the derivatizing agent, FMOC-Cl, was 20 mM. The reverse effect was observed when the FMOC-Cl derivatizing agent was decreased; the 5% borate performed significantly higher than the 10% borate addition. This indicates a proportional relationship between the two factors and underlines the need for borate addition to ensure the complete derivatization of the target compounds.

4. VALIDATION

The method performance parameters obtained for the pre-column derivatization procedure are shown in Table 3. Linearity was confirmed from the evaluation of the residual distribution, and coefficients of determination (R^2) were ≥0.98 (Figures S7−S12). Statistical evaluation of the results showed that intercept values were not significantly different. Repeatability and interday precision, namely, relative standard deviation (RSD) <4.5% at the low level and <3.8% at the high level, were considered acceptable. No target compounds were detected in the blank controls. Table 3 also includes the LODs and LOQs. The method limits were obtained from instrumental limits using an analytical process, and as the S/N is an instrumental LOD, they do not significantly increase the variability and bias of analytical data. The outcomes show that

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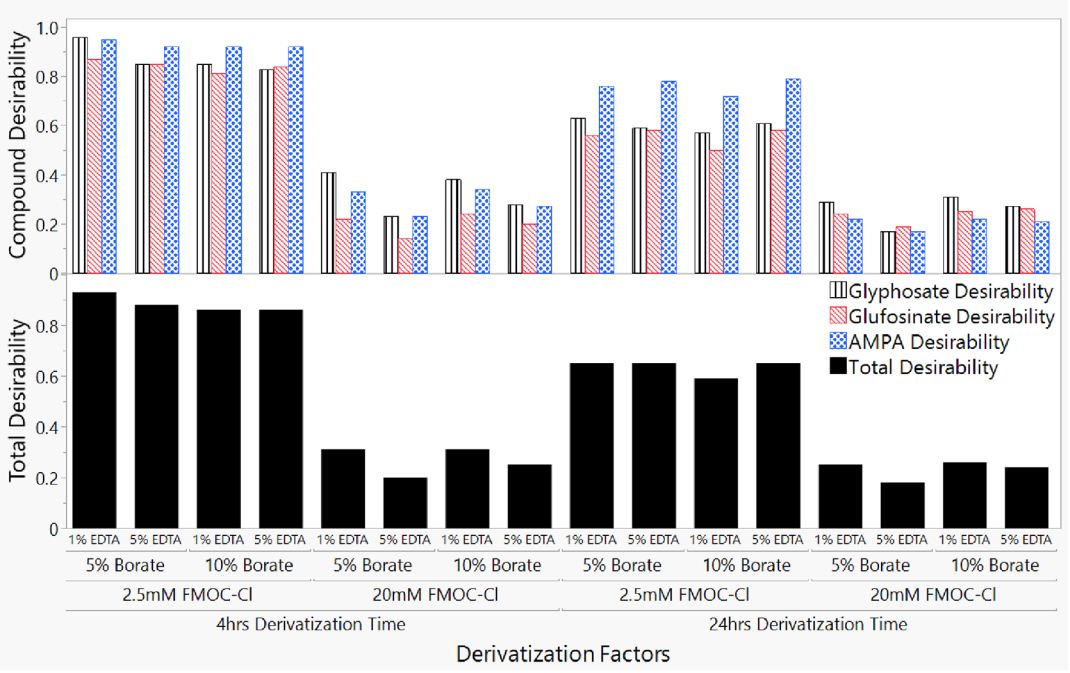


Figure 7. Desirability of optimal conditions for the complete derivatization of glyphosate, glufosinate, and AMPA in terms of reaction time and derivatizing agent concentration.

Table 3. Validation Parameters for the Optimized Derivatization Method

	precision (RSD, %) $n = 3$							
	repeatability		interday		limits (0.01−1 μg/L)		calibration (0.01−1 μg/L)	calibration (1−100 μg/L)
compounds	low	high	low	high	LOD	LOQ	R^2	
glyphosate	3.50	2.10	3.70	0.60	0.04	0.12	0.989	0.997
glufosinate	1.20	0.70	1.10	0.10	0.03	0.09	0.994	0.997
AMPA	4.40	3.70	2.30	1.10	0.05	0.16	0.981	0.995

the derivatization reaction significantly reduces the findings' substantial variability, which must be considered. The quantification limits for the examination of waters are near the European drinking water quality regulations, which establish a parametric value for pesticides of 0.1 μg/L,[6] a number that is frequently surpassed while monitoring surface waters. To obtain detections below the EU regulations and requirements for this application, a post-derivatization concentration and reconstitution process would be necessary, even though this has no bearing on the optimal derivatization conditions discovered in this work.

5. CONCLUSIONS

This study developed and optimized an accelerated and simplified method of quantifying glyphosate, glufosinate, and AMPA using FMOC-Cl derivatization. Although this matrix is complex and challenging, optimizing the chromatographic and experimental parameters produced a rapid, sensitive, and accurate assay. The assessed parameters included borate and EDTA buffer addition, FMOC-Cl concentration, and derivatization time. Complete reactivity with high sensitivity was achieved with 2.5 mM FMOC-Cl after 4 h. Higher concentrations of FMOC-Cl produced byproducts generated from the reaction between water and amino acids in sample matrices caused analytical interference and must be separated from the targeted analytes. The method was validated to meet all the requirements of selectivity, linearity, lower limit of quantitation, matrix effects, and stability. After derivatization, the optimized method, despite the complexity, was sufficiently sensitive and precise to quantify glyphosate and AMPA residues in water. The complexation and metallic interaction with target chemicals, varying retention times, and reduced glyphosate, glufosinate, and AMPA sample sensitivity were resolved. This work highlighted the required selectivity and sensitivity for the trace level measurement of glyphosate, glufosinate, and AMPA due to their ionic and polar characteristics.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.3c00094.

Chromatographic responses for targeted compounds, interaction profiles, calibration curves for borate, EDTA, FMOC, and the interaction between various derivatization factors (PDF)

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Notes

The authors declare no competing financial interest.

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CHAPTER 3

IMPROVED SIMULTANEOUS QUANTIFICATION OF GLYPHOSATE-BASED HERBICIDES IN URBAN WATERSHEDS IN CALIFORNIA

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This chapter is under review at a peer-reviewed journal. This chapter addresses research question 1) How do novel detection techniques and analytical tools contribute to enhancing our ability to monitor glyphosate residues in various water sources, and what are the implications of these findings for environmental and public health management?

Abstract

Urgent development of analytical tools is necessary to address the intricate relationships among agriculture, water scarcity, and the extensive application of herbicides. These tools aim to achieve a balance between agricultural productivity and water conservation, all the while ensuring the preservation of water quality. This study investigated techniques for enhancing sample matrix enrichment, and enhancing acquisition protocols for glyphosate, glufosinate, and aminomethylphosphonic acid (AMPA). Offline solid-phase extraction (SPE) combined with liquid chromatography tandem mass spectrometry (LC-MS/MS) improved

both selectivity and sensitivity in the analysis. The objective was to attain limit of detection (LOD) and limit of quantification (LOQ) that exceeded the maximum residue limits set by the World Health Organization (0.1 ppb). This study attained detection limits that were 10 times lower than the WHO limit. Under improved experimental conditions, analyte recovery ranged from 80% to 95%, with LOQs between 37 to 55 ng/L. The most effective eluent for recovering all analytes from the sorbent was 100% MeOH using an Oasis MCX cartridge, with the flow rate at 0.3 mL/min and a sample volume of 2 mL showing proportional effects on analyte recovery post SPE. This streamlined LC-MS/MS coupled with SPE method for routine testing of glyphosate, glufosinate and AMPA in water promises to enhance environmental and public health studies concerning the usage, drift, and exposure to these pesticides.

Keywords: pesticide, organophosphates, SPE, LC-MS/MS

Introduction

California is a global agricultural powerhouse and is at a critical juncture, wrestling with the complex task of balancing farming, water scarcity, and pesticide use– particularly glyphosate and glufosinate, amidst persistent droughts. Urgently, a comprehensive strategy that includes advanced analytical tools to accurately monitor and mitigate glyphosate's impact on water resources is needed [85, 86]. The adoption of broad-spectrum, nonselective, post-emergence pesticides like glyphosate and glufosinate, driven by the rise of resistant crops, has resulted in the transportation and pervasive presence of these pesticides in water resources [63, 87]. Glyphosate and its metabolite, aminomethylphosphonic acid (AMPA), are often found in surface and ground waters despite expectations of soil absorption [1, 58], raising environmental and health concerns leading to ongoing regulatory scrutiny exists for these pesticides, with some jurisdictions considering bans or restrictions [22, 81, 88].

Reliable and efficient analytical technologies are crucial for environmental research on glyphosate and glufosinate, requiring excellent performance in selectivity, detectability, and sensitivity. The difficulty in quantifying glyphosate-based compounds at sub-µg/L levels is attributed to their ionic nature and the absence of identifiable chemical groups for traditional detection methods [43]. Analytical techniques such as gas chromatography (GC) [71], high-performance liquid chromatography (HPLC) [89, 90], capillary electrophoresis (CE) [91], and enzyme-linked immunosorbent assay (ELISA) [3, 32] have been used for glyphosate determination. However, liquid chromatography-mass spectrometry (LC-MS/MS) is favored for simultaneous quantification of glyphosate, glufosinate, and AMPA [92], requiring derivatization with 9-fluorenyl-methyl chloroformate (FMOC-Cl) to enhance

hydrophobicity and molar mass [14, 93]. The Limit of Quantification (LOQ) for glyphosate varies depending on the technique used. GC and CE methods typically have LOQs ranging from 0.1 to 0.8 ppm [33], while HPLC methods commonly yield LOQs of 50 ppb [94]. ELISA assays usually provide higher LOQs, typically around 1 ppm [33].. In contrast, LC-MS/MS methods offer the lowest LOQs, often reaching levels as low as 0.6 ppb. LC-MS/MS provides versatility in handling complex sample matrices with high quantitative accuracy, making it widely accepted for pesticide residue analysis [14].

Concerns persist regarding sensitivity and matrix effects in existing methods, attributed to the absence of a concentration step, despite derivatization benefits [43]. Solid-phase extraction (SPE) is identified as effective for analyte enrichment and interference removal, addressing issues related to method efficiency and laborious sample preparation [17]. Online and offline enrichment strategies, including SPE discs and ion-exchange sorbents, demonstrate significant improvements in analyte recovery and matrix enhancement [92, 95, 96]. However, challenges related to reproducibility and matrix effects make online SPE cumbersome for the accurate quantitative characterization of glyphosate, glufosinate, and AMPA in water matrices due to the potential for cross-contamination [97, 98]. This study aims to develop an efficient method utilizing offline SPE and LC-MS/MS for the quantification of glyphosate, glufosinate, and AMPA in environmental waters, emphasizing accurate measurements with low detection limits, reduced matrix effects, and high throughput. Outcomes of this study addresses challenges associated with 1) herbicide analysis to mitigate environmental and health consequences, 2) persistence of these pesticides in environmental surface water samples, and 3) improving sample preparation

and analytical conditions to enhance selectivity and sensitivity, contributing to the understanding and management of herbicide contamination in water.

Materials and Method

Chemicals and reagents

Glyphosate (99%), AMPA (99%), and glufosinate-ammonium (99%) were obtained from Chemservice (West Chester, PA). In deionized (DI) water, primary stock solutions were made with purity-adjusted concentrations of 1 g/L for all compounds. A working solution with 10 µg/L concentrations of glyphosate, glufosinate, and AMPA was made and kept at 4 °C. A series of spiked solutions comprising all target analytes were created, ranging in concentration from 0.5 to 1 µg/L to fortify samples and create calibration standards. An internal standard solution was prepared in DI water with a concentration of 50 µg/L from 1,2-$^{13}C_2{}^{15}N$ glyphosate (99%) and $^{13}C^{15}N$ AMPA (99%) isotopically labeled standards acquired from Cambridge Isotope Laboratories (Andover, MA). LC-MS grade water, ethylenediaminetetraacetic acid (EDTA), acetonitrile (ACN), formic acid (CH_2O_2), sodium tetraborate, 9-fluorenyl-methyl chloroformate (FMOC-Cl), methanol (MeOH), ammonium acetate ($C_2H_7NO_2$), ammonium hydroxide (NH_4OH) and phosphoric acid (H_3PO4) were obtained from Fischer Scientific (Hampton, NH).

Instruments and configuration

The study used an Agilent (Palo Alto, CA) Series 1290 LC coupled with an Agilent 6470A triple quadrupole mass spectrometer. The setup included a binary pump, column

oven, and autosampler. Electrospray ionization was used in negative mode to introduce analytes into the mass spectrometer. The drying gas flow rate was set at 5 L/min at 300°C, with a nebulizer pressure of 45 psi and a fragmentor voltage of 110 V. Agilent Masshunter Qualitative and Quantitative software was used for processing the chromatograms. A Phenomenex Gemini NX-C18 column (particle size: 3 m; length: 100 mm; internal diameter: 2.1 mm) with a solvent flow rate of 0.3 mL/min was used. Each injection contained a 20 µL matrix/sample and the column compartment temperature was kept at 30 °C at a concentration of 1 mM $C_2H_7NO_2$ in LC grade water constituted mobile phase A, while ACN made up mobile phase B. The mass transition ion pairs for the target ions and LC mobile phase gradient are shown in Table 3.1. The LC conditions were enriched to ensure high sensitivity and selectivity of the target compounds.

Table 3.1. LC gradient and mass spectrometry transition parameters.

Run Time	9 min		
LC Gradient	Time(min)	% Mobile Phase A	% Mobile Phase B
	0	90	10
	2	90	10
	8	60	55
	8.1	90	10
	9	90	10
Mass Spectrometry Transitions	Target Analytes and Isotopes	Quantifier	Qualifier
	Glyphosate FMOC-Cl	389.9 → 168	389.9 → 63
	Glufosinate FMOC-Cl	402.1 → 180	402.1 → 206
	AMPA FMOC-Cl	332.1 → 110	332.1 → 136
	Isotope labeled $+2H_4$-AMPA FMOC-Cl	336 → 114	
	Isotope labeled $+3H_4$-glyphosate-FMOC-Cl	391.9 → 170	

Preparation of standard solution

Precise and reliable primary standard solutions were meticulously prepared for glyphosate, AMPA, and glufosinate by weighing 10 mg of each standard, corrected for purity, into individual 10 mL volumetric flasks and dissolving them in deionized (DI) water. These solutions, with a concentration of 1 mg/mL, served as accurate references for subsequent

analyses. Additionally, a working standard mix was created in an amber plastic container by combining 0.1 mL of each 1 mg/mL standard, resulting in a 1 µg/mL solution containing all three standards—glyphosate, AMPA, and glufosinate. The total volume was adjusted to 97 grams (g) using DI water, ensuring uniformity. To maintain stability, all solutions are stored at 4 ºC, preserving their integrity and reliability for future analytical use. This meticulous preparation and storage process ensured consistency and accuracy in determining the concentrations of glyphosate, AMPA, and glufosinate in subsequent environmental water analyses.

Collection and preparation of samples for LC-MS/MS analysis

Seventeen water samples were collected across several sites in California from October to November 2023. Seven surface and groundwater samples were obtained from the San Joaquin Wildlife Sanctuary in Irvine, CA, which is operated by the Irvine Ranch Water District (IRWD). The sanctuary, which covers approximately 80 acres, is designed to protect wildlife from harmful runoff from incoming San Diego Creek water. The sanctuary includes holding ponds for pre-screening, irregularly shaped ponds for nitrate removal and avian habitat, and a polishing pond for particle removal. The marsh effluent is discharged into San Diego Creek, which flows into Newport Bay and eventually the Pacific Ocean [99]. The marsh serves as a reservoir for recycled water from IRWD Michelson water reclamation plant, used for irrigation, cooling towers, dust control and industrial processes [100]. Ten tap water samples were collected from Orange (6), Sacramento (2) and San Luis Obispo (2) counties. The sample collection in Sacramento County was designed to assess water quality in

suburban with a focus on understanding the impact upstream and nearby agriculture on water resources. The Orange and San Luis Obispo samples provide insight into the dynamics and prevalence of the target analytes in both coastal and inland settings, considering the influence of urbanization. The grab samples were filtered with 0.45 μm glass fiber filter and derivatized to avoid degradation of target analytes. These sample collection efforts contribute to a comprehensive understanding of water quality across diverse regions, supporting environmental monitoring and management strategies.

The detailed classification and physiochemical information of these target analytes are highlighted in Table S3.1. The derivatization protocol used for this study was adapted from Martin et. al [14]. Ten mL were transferred into an amber glass bottle, supplemented with 200 μL of the isotope internal standard solution to mitigate matrix effects. 0.5 mL each of 5% (w/v) borate buffer and 1% (w/v) EDTA solution, followed by 1.5 mL of the 2.5mM FMOC-Cl stock solution was then added. Subsequently, the samples were incubated in a water bath at 40 °C in darkness for 4 hours. Following this, 0.6 mL of H_3PO4 solution (2% v/v) was added, and the samples were kept at 4 °C until SPE, with storage maintained in the absence of light. For SPE, a BTLab (St. Louis, MO) nitrogen sample concentrator was used in conjunction with an extraction manifold from Waters (Milford, MA). This manifold has three interlocking mechanisms and twenty positions, ensuring ideal sample preparation and proper cartridge placement for effective collection. The offline pre-concentration and extraction were accomplished using a variety of Oasis SPE polymeric based phase sorbents from Waters (Milford, MA) - Oasis MAX, Oasis MCX, Oasis WCX, Oasis WAX and Oasis HLB. The efficiency of the mixed-mode sorbent and the ability of different eluents to recover

glyphosate, glufosinate and AMPA were investigated. An aliquot of each derivatized sample was combined in a test tube. Two mL of MeOH was added to the cartridge for conditioning (Table 3.2). The cartridge was washed with 2 mL of DI water for equilibration then 2mL of the derivatized sample was loaded onto it at a rate of 1mL/min. The washing of interferences and elution of the analytes was accomplished at a rate of 1 mL/min using organic solvents. A nitrogen evaporator was then used to dry the eluates, which were subsequently reconstituted and subjected to chromatographic analysis. Extraction efficiency parameters such as elution volume and composition, cartridge conditioning effect and wash volume were investigated to enhance chromatographic sensitivity and selectivity. The newly developed analytical method with SPE was applied on grab samples and results were compared to the derivatization only method outlined in Martin et al.'s study [14].

Table 3.2. SPE Conditions for Various Cartridges.

<table>
<tr><td></td><td>Oasis HB</td><td>Oasis WAX</td><td>Oasis MCX</td><td>Oasis MAX</td><td>Oasis WCX</td></tr>
<tr><td>Conditioning (2ml)</td><td colspan="5">100% MeOH</td></tr>
<tr><td>Equilibration (2mL)</td><td colspan="5">DI water</td></tr>
<tr><td>Loading (2 mL)</td><td colspan="5">Analyte in water matrix</td></tr>
<tr><td>Washing (1 mL)</td><td>5% MeOH</td><td colspan="2">2% Formic acid</td><td colspan="2">5% NH_4OH</td></tr>
<tr><td>Elution (4ml)</td><td>85%-15% ACN-MeOH</td><td>5%-95% NH_4OH - MeOH</td><td>100% MeOH</td><td colspan="2">2% CH_2O_2 in MeOH</td></tr>
</table>

LC Method Optimization

The effective SPE conditions were obtained after improvement of several LC parameters. This study compared the effectiveness of two analytical columns brands, Phenomenex Gemini and Agilent Eclipse, with different column lengths (50 mm and 100mm for both) in quantifying target analytes. Phenomenex Gemini columns have dual functionality, having both C18 and polar embedding groups, making them versatile in compound separation and applicable to a wide range of analytes and sample matrices [101]. Agilent Eclipse columns, on the other hand, use a more conventional C18 stationary phase, which is known for its durability and dependability, resulting in consistent performance and outstanding peak forms in chromatographic separations [102]. A comparative analysis and evaluation were derived based on the chromatographic efficiency of each column. The temperature, flow rate, mobile phase acidification and gradient were refined to enhance selectivity and sensitivity in detection of glyphosate, glufosinate and AMPA (Table 3.3).

Table 3.3. Summary of parameters assessed for LC analysis.

Parameters	**Variables**
Temperature (^{o}C)	30, 40 and 50
Mobile phase B gradient (%)	10-40, 10-60, and 10-90
Mobile phase A acidification (mM)	0.1, 1.0 and 5
Column flowrate (mL/min)	0.3, 0.4, and 0.5

Method Validation

Target analytes were identified based on criteria such as retention time deviation, mass/charge ratios, and relative ion ratios. Method precision and recovery were assessed by analyzing fortified blank samples, while method linearity was confirmed through duplicate testing of seven standard solutions. Triplicates were employed for each calibration level. The limit of detection (LOD) and limit of quantification (LOQ) were determined across a range of sample and standard spikes (1 to 10 µg/L) in DI water, ensuring accuracy and precision. The LOD and LOQ for each analyte in two matrices were established as the concentrations yielded signal-to-noise ratios (S/N) of 3 and 10, respectively, with analyte concentrations adjusted to meet these S/N criteria.

The method's linearity was further verified with duplicate testing of seven standard solutions, and precision and recoveries were evaluated through the analysis of fortified blank samples. Isotopically labeled standards had a limit of 50 µg/L. Triplicates were used for each calibration level. Matrix effects were determined by comparing peak areas in LC water and matrices spiked with 50 ng/L for each analyte (Equation S3.1). Selectivity was assessed for potential internal substance interferences using separate batches of spiked solutions. Carryover was evaluated by injecting a blank solution after the highest control standard. If the resulting area was less than 20% of the LOQ for target analytes, it was considered free from interfering components, and the blank sample area following the highest control standard was accepted. To assess the performance difference between sample pre-treatment via FMOC-Cl derivatization and sample concentration via SPE, statistical analysis was conducted in JMP statistical software (Cary, NC). ANOVA and

Wilcoxon test were utilized for multilevel analysis. The results were graphically represented using Origin (Northampton, MA).

Results and Discussion

Improved detection and quantification of glyphosate-based herbicides

The remarkable achievement of detection and quantification limits (LOQ) exceeding the World Health Organization's (WHO) maximum residue limits for glyphosate, glufosinate, and AMPA in water samples was realized through meticulous method optimization (Table 3.4). By systematically refining experimental conditions, including SPE protocols, chromatographic separation techniques, and derivatization steps, the study successfully attained detection limits that were tenfold lower than the WHO threshold. Notably, this optimization effort resulted in robust analyte recovery rates ranging from 80% to 95%.

The fine-tuning of parameters such as sorbent phases, elution solvents, and loading volumes contributed to the achievement of LOQs between 37 to 55 ng/L, signifying a significant advancement in sensitivity and precision. By meticulously optimizing each step of the analytical process, from sample preparation to detection, the study exemplifies the importance of methodological refinement in surpassing regulatory standards and enhancing the accuracy and reliability of pesticide residue analysis in water samples. These improved limits not only enable retrospective analysis of samples from previous studies with greater accuracy but also enhance ongoing monitoring efforts by providing more robust and reliable detection of pesticide contamination at lower concentrations. The implications extend to

proactive management strategies, facilitating more effective safeguarding of water quality and public health.

Table 3.4. Comparison of methods for the determination of glyphosate, glufosinate and AMPA in water samples, denoting if the derivatization was performed before or after the SPE step.

Sorbent phase	**Separation technique**	**Derivatization**	**Compound**	**LOD (µg/L)**	**LOQ (µg/L)**	**Reference**
Oasis MCX	LC-MS/MS	FMOC-Cl (before)	Glyphosate Glufosinate AMPA	0.015 0.012 0.018	0.045 0.037 0.055	This method
MIP-SBSE	LC-FAD	FMOC-Cl (before)	Glyphosate Glufosinate AMPA	0.150 - -	0.468 - -	[103]
Grace Maxi-Clean™	LC-MS/MS	-	Glyphosate Glufosinate AMPA	- - -	3.04 - 5.06	[104]
Oasis HLB	UHPLC-MS/MS	FMOC-Cl (before)	Glyphosate Glufosinate AMPA	0.2 - 0.2	0.6 - 0.6	[105]

Enhanced Liquid chromatography conditions

Shorter column lengths (both Phenomenex and Agilent) demonstrated improved sensitivity ($p<0.05$); however, the Phenomenex Gemini 100mm exhibited superior selectivity due to longer retention times and increased analyte interaction with the stationary phase ($p<0.05$), providing optimal conditions for separation (Figure S3.1). The hybrid phases of Phenomenex Gemini columns, combining characteristics of C18 and C8 stationary phases providing unique selectivity and stability for the analytes, outperformed both Agilent Eclipse columns [106]. Lowering the column temperature to 30°C significantly improved the retention time, selectivity, and sensitivity of the target analytes (Figure S3.2) ($p < 0.05$). The improved sensitivity was as a result of reduced baseline noise and minimized analyte peak broadening when compared to 40 and 50 ^{o}C transitions ($p<0.05$) [107, 108]. The influence of the organic mobile phase, ACN, revealed that a lower gradient ensured optimal interaction with the stationary phase, resulting in better peak shapes and higher signal-to-noise ratios. Increasing the gradient can increase the efficiency of separation, but it is important to balance the trade-offs between retention and selectivity [109]. Higher organic gradient led to signal suppression due to reduced ionization efficiency and co-elution of the analytes [12] (Figure S3.3).

Acidification of mobile phase A with 1 mM $C_2H_7NO_2$ exhibited the optimal selectivity compared to 0.1 mM and 5 mM, enhancing the retention of compound targets ($p<0.05$) (Figure S4). The detection of glyphosate in a 0.1mM transition showed ghost peaks but an increase to 5 mM $C_2H_7NO_2$ concentration led to ion suppression due to pH and hydrophobicity changes. This increased pH in the stationary phase was not suitable for

compound targets retention. Acidification of the mobile phase increased compound retention, resulting in increased affinity for ionized functional groups on the stationary phase [110]. Additionally, a reduced flow rate of 0.3 mL/min enhanced sensitivity and retention, proving advantageous for compounds with closely eluting peaks, leading to improved separation quality (Figure S3.5). The higher flow rate resulted in lower compound response, as compounds move too quickly for detection. Column flow rate is directly proportional to retention time in LC, while slower flow rates lead to extended retention times and enhanced selectivity, resulting in heightened peak resolution and improved separation quality [111]. These findings contributed to the development of an enhanced LC-MS/MS method for precise analysis of glyphosate, glufosinate, and AMPA in environmental waters.

Cartridge selection and extraction efficiency

While the Oasis HLB cartridge yielded recoveries below the acceptable range [112], Oasis MCX and Oasis WAX cartridges demonstrated significant improvements, with recoveries exceeding 86% and 80% respectively for most analytes (Figure S3.6) (Table 3.5). The superior performance of Oasis MCX cartridges was attributed to their mixed-mode cation exchange chemistry, facilitating effective extraction [113]. Conversely, Oasis MAX and Oasis WCX cartridges performed poorly due to their hydrophobic and cation/weak exchange functionalities, which inadequately interacted with the target compounds' ionizable functional groups [112, 113]. These findings highlight the importance of selecting the appropriate SPE cartridge for optimal recovery rates in glyphosate analysis.

Table 3.5. Recoveries of different SPE cartridges using conditions in Table 1.

		Recovery (%)		
Elution Solvent	**Cartridge**	**Glyphosate**	**Glufosinate**	**AMPA**
100% MeOH	Oasis MCX	85 ± 0.11	95 ± 0.34	85 ± 0.22
5% NH_4OH in MeOH	Oasis WAX	70 ± 0.47	80 ± 0.91	83 ± 0.47
85/15% ACN/MeOH	Oasis HLB	60 ± 0.95	60 ± 1.2	53 ± 2.4
2% CH_2O_2 in MeOH	Oasis MAX	42 ± 1.9	43 ± 2.1	46 ± 1.6
2% CH_2O_2 in MeOH	Oasis WCX	24 ± 0.56	25 ± 0.47	27 ± 0.95

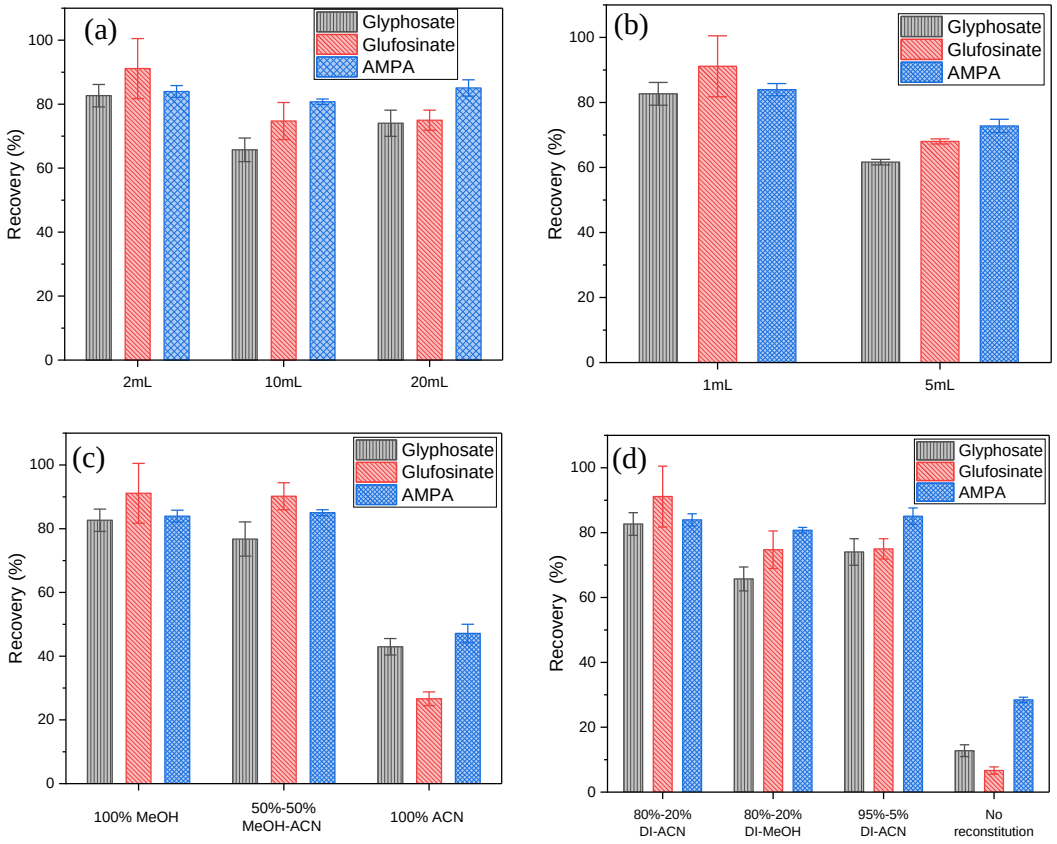


Figure 3.1. Effects of (a) loading volume, (b) washing volume with 2% Formic acid, (c) elution solvent composition with 4 mL volume, and (d) matrix reconstitution on the chromatographic separation of analytes

Conditioned cartridges exhibited significantly higher recovery rates for all analytes compared to unconditioned cartridges ($p<0.05$), with recoveries exceeding 80% for conditioned cartridges and dropping below 70% for unconditioned ones (Figure S3.7). Conditioning with organic solvents optimized sorbent interaction with target analytes, improving reproducibility and avoiding undesirable sample-sorbent interactions. Using the lowest loading volume of 2 mL resulted in higher recoveries for glyphosate, glufosinate, and AMPA, with significantly lower recoveries by 10-20% with increased loading volumes ($p<0.05$) (Figures 3.1a and S3.8). Lower flowrates (1 mL/min) improved target analyte recoveries compared to higher flowrates (5 mL/min), while increased washing volumes (5 mL) led to significant losses in recoveries ($p<0.05$) (Figures 3.1b and S3.9). Elution with 100% MeOH provided the best recovery for polar compounds, with poor recoveries observed when using only ACN, while a combination of MeOH and ACN (50%-50%) improved recoveries and reduced matrix effects (Figure 3.1c and S3.10). Sample reconstitution with a combination of ACN or MeOH with DI water significantly enhanced target analyte recovery ($p<0.05$), with 20% ACN in DI water achieving improved recoveries >80%, ensuring sample integrity and proper concentration adjustment for improved sensitivity and reduced matrix effects in downstream analyses (Figure 3.1d).

Quality Control, Method Performance and Validation

Two different water matrices were assessed to evaluate the recovery accuracy at four spiking levels. Recoveries of 90-98% were achieved for target analytes at spike levels of 2.5

µg/L, 5 µg/L and 10 µg/L (Table S3.8). At spike level of 1 µg/L, the recoveries ranged from 88-93%. To assess linearity, ultrapure water was spiked at concentrations ranging from 0.1 to 10 µg/L. Table 3.6 showed that all standard curves (Figure S3.11 - S3.13) for the target analytes exhibited coefficients (R^2) > 0.98. The method, which incorporates internal calibration, underwent validation by assessing repeatability, LODs, and LOQs. Repeatability was calculated by determining the relative standard deviation of the analyte quantification.

Table 3.6: Validation parameters for optimized offline SPE method Relative standard deviation (RSD)].

Compounds	**Precision (RSD, %) n=3**		**Limits (ng/L)**		**Calibration (0.01-1 µg/L)**
	Repeatability	**Interday**	**LOD**	**LOQ**	**R^2**
Glyphosate	1.12	0.87	15	45	0.99
Glufosinate	0.93	0.56	12	37	0.99
AMPA	1.35	0.97	18	55	0.98

Method Application in Grab Samples from surface water

None of the samples collected and examined showed the presence of glufosinate regardless of the method used (Figure 3.2). However, when utilizing the SPE method, glyphosate and AMPA were detected in all samples, whereas only glyphosate was detected without using SPE. The differences in polarity and hydrophobicity between glyphosate and

AMPA during SPE may have affected their retention on the chromatographic column, further contributing to increased detection sensitivity and ionization efficiency between the two compounds [106].While concentrations of glyphosate without SPE are higher, it is overestimating the concentrations of glyphosate without the clean-up process that SPE provides. The formation of FMOC-OH during glyphosate derivatization, which can lead to false positives due to its potential interference with the detection system [14], could contribute to the increased signal. Therefore, this finding validates the adoption of SPE in the analysis of glyphosate, glufosinate and AMPA. Potential matrix interferences were removed and hence improved the selectivity and sensitivity of the chromatographic detection.

All sampled sites exceeded the World Health Organization (WHO) limit for allowable glyphosate residue of 0.1 ppb [3]. The outlet of the marsh exhibited the highest concentration of glyphosate and AMPA, indicating a potential for adverse environmental and public health impacts. Additionally, higher concentrations from the outlet of the marsh could indicate water treatment and wastewater treatment plants as a source of glyphosate residue into the environment as described in previous studies [1]. Therefore, managing and reducing pesticide contamination in watersheds is important for protecting water quality and ecosystem health [63]. The higher glyphosate concentrations and lower AMPA concentrations detected in the sampling points indicate a combination of either slower degradation, sampling closer to application time or faster transport connectivity [22, 114]. Monitoring both glyphosate and AMPA provides a comprehensive understanding of glyphosate presence and fate in water systems.

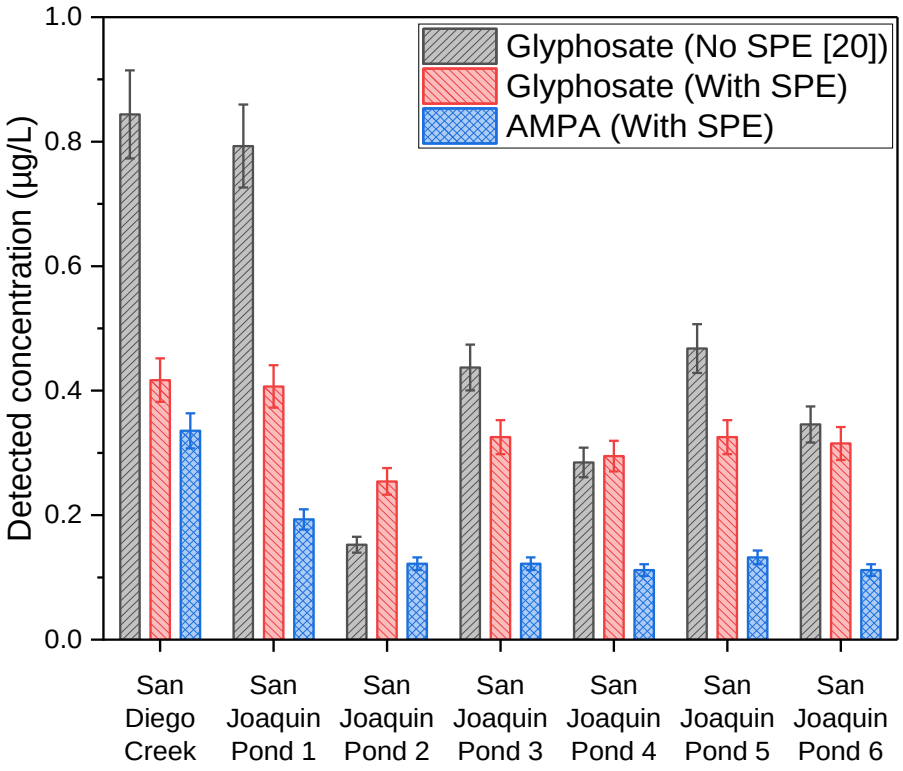


Figure 3.2. Summary of detected concentrations of target analytes in grab samples.

Glyphosate, glufosinate and AMPA in tap water.

Out of the ten tap water samples, glyphosate and AMPA were detected in 8 samples whereas glufosinate was not detected in any of the samples (Table 3.7). The two non-detections of glyphosate and AMPA were found on the samples collected from Orange County within Irvine Ranch Water District. The Orange County samples exhibited higher concentrations compared to those from Sacramento and San Luis Obispo, likely influenced by the county's higher urbanization, population density, and increased pesticide usage for landscaping and weed control [115] . Proximity to cultivated areas and potential downstream agricultural runoff further contributes to elevated pesticide concentrations, along with variations in land use, pesticide application practices, stormwater runoff, water

management, climate conditions, and human activities. These factors collectively influence the detection levels of glyphosate and AMPA in Orange County relative to Sacramento and San Luis Obispo.

Table 3.7. Averaged results of detected target analytes in sampled tap water.

County	**Glyphosate (µg/L)**	**Glufosinate (µg/L)**	**AMPA (µg/L)**
Sacramento	0.14 ± 0.09	-	0.10 ± 0.09
San Luis Obispo	0.11 ± 0.06	-	0.11 ± 0.06
Orange	0.21 ± 0.08	-	0.16 ± 0.09

Conclusion

The development of a streamlined offline solid-phase extraction (SPE) coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS) approach, requiring minimal pretreatment, holds profound implications in the field of glyphosate-based herbicide analysis. By achieving detection limits that are 10 times lower than the WHO limit of 0.1 ppb and well below existing methods with reported lowest limits of 0.4 ppb, this study represents a significant advancement in our ability to accurately assess glyphosate, glufosinate, and AMPA in ambient waters. The emphasis on optimizing various parameters underscores the importance of meticulous method development to achieve accurate measurements with minimal matrix interference. This breakthrough not only enhances our understanding of herbicide contamination in aqueous environmental matrices but also provides a powerful tool for regulatory compliance and environmental monitoring,

ultimately contributing to the management and mitigation of glyphosate-related risks to ecosystems and public health.

The method achieved high accuracy, precision, and low quantification limitations, making it well-suited for the high-throughput determination of glyphosate, glufosinate, and AMPA in environmental waters. Key findings include the optimization of column temperature at 30°C, the importance of mobile phase composition with a lower gradient of acetonitrile, the influence of ammonia acetate concentration on selectivity, and the significance of flow rate, loading volume, washing, and elution in achieving high recoveries. Oasis MCX, chosen from a selection of cartridges with varying retention and recovery capacities, demonstrated proficiency in capturing the target compounds. The developed approach provides numerous benefits, including a streamlined process that improves reproducibility and minimizes solvent consumption. Additionally, the method optimally utilizes a small sample volume, leading to significantly increased efficiency in both pretreatment and analysis processes. The study notably found higher concentrations in Orange County samples compared to those from Sacramento and San Luis Obispo, with factors such as urbanization, population density, and heightened herbicide usage in California playing significant roles. This developed offline method is well-suited for the high-throughput determination of glyphosate, glufosinate, and AMPA in environmental waters, providing valuable insights into advancements in quick monitoring methods marked by high efficiency and cost-effectiveness.

CHAPTER 4

EXPLORING PESTICIDE TRANSPORT, GROUNDWATER, AND ENVIRONMENTAL JUSTICE IN A CHANGING CLIMATE: A COMMUNITY ENGAGED RESEARCH APPROACH

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This chapter is under review at a peer-reviewed journal. This chapter addresses research questions 1) How do novel detection techniques and analytical tools contribute to enhancing our ability to monitor glyphosate residues in various water sources, and what are the implications of these findings for environmental and public health management?

2) What are the spatiotemporal dynamics governing the transport and distribution of glyphosate, glufosinate, and AMPA within watersheds, and how can remote sensing technologies and groundwater interconnectivity models inform strategies for mitigating environmental impacts? and 3) What socio-economic and environmental justice factors influence the interplay between agricultural practices, glyphosate usage, and societal equity considerations, and how can this understanding inform the development of sustainable management practices tailored to diverse rural and urban landscapes?

Abstract

The pressing issue of pesticide exposure disproportionately affecting marginalized communities underscores the immediate necessity to tackle pesticide drift from nearby agricultural areas, especially aggravated by the impacts of climate change. Effective measures including stricter regulations, enhanced monitoring, alternative agricultural practices, and community engagement are essential to mitigate environmental injustices and safeguard community health. This article delves into the intricate relationship between pesticide transport, groundwater vulnerability, and environmental justice within the context of climate change. Employing a geospatial analytical hierarchy overlay model, we comprehensively assess the impact of pesticide transport on groundwater vulnerability while scrutinizing climate change and associated environmental justice concerns. Groundwater vulnerability across the basin varies, with 18% classified as very low, 23% as low, 27% as prone, and 20% and 12% as high and very high, respectively, concentrated mainly in the mid-eastern and southern regions due to population density and biodiversity. The research integrates a robust analytical detection technique, with a focus on glyphosate and its metabolites concentrations, to validate and refine spatial models. By engaging with communities, this study enhances understanding of environmental complexities, offering insights for sustainable environmental management.

Keywords: Glufosinate, Glyphosate, Aminomethylphosphonic acid (AMPA), Sustainability, Geospatial Modeling, Environmental Policy, Analytical Detection, Environmental Complexities, Aquifer Vulnerability

Introduction

Non-point source runoffs such as glyphosate and glufosinate contaminate surface and groundwater used for drinking [1]. Glyphosate and glufosinate are nonselective post-emergence alternatives to traditional organochlorine pesticides due to resistant crop varieties in minimizing crop losses caused by harmful organisms and pests [5, 29, 87]. Glyphosate and its metabolite, aminomethylphosphonic acid (AMPA), are frequently detected in surface and ground waters due to widespread use in agriculture, industry, and households, increasing the risk of environmental toxicity [3, 22]. Poiger, et al. [116] detected the presence of glyphosate in the effluent of wastewater treatment plants highlighting glyphosate persistence after remediation and contributions to surface water. Additionally, a significant portion of Kentucky's drinking water is sourced from surface and groundwater supplies, the presence of glyphosate contamination raises notable concerns for effective monitoring tools and protocols [117].

Researchers have developed methods to quantify glyphosate, glufosinate, and AMPA levels in surface and groundwater, addressing the limitations in regulatory benchmarks [14]. Traditional technologies like gas chromatography are used, but recent advancements have combined derivatization techniques with liquid chromatography tandem mass spectrometry (LC-MS/MS) [11, 70, 90]. The use of LC-MS/MS improves the sensitivity and selectivity of detecting glyphosate, glufosinate, and AMPA in water [33]. A previous study showcased a robust pre-column detection method using LC-MS/MS, achieving quantification limits of 0.12 μg/L [14]. This method allows for accurate measurement and monitoring of trace levels of

glyphosate, glufosinate, and AMPA, which is critical for assessing the vulnerability of surface and groundwater sources.

Geospatial modeling via Saaty's Analytical Hierarchy Process (AHP) reveals a significant correlation between groundwater vulnerability, climate change-induced pesticide transport, and challenges to marginalized populations' quality of life [6]. Groundwater vulnerability is a pressing concern due to rapid agriculture production, population growth, and climate change's impact on water cycle and runoff from intensified rains [118]. Unpredictable climate change patterns contribute to increased pesticide transportation through runoff and leaching, which disproportionately affects marginalized areas [119]. Due to their closeness to agricultural districts with significant pesticide usage, Black, Indigenous, and People of Color (BIPOC) and economically marginalized populations are frequently the most affected [120]. Rising precipitation and climate-related floods can transport pesticides, potentially contaminating groundwater and increase citizens' concerns of water quality and perpetuating environmental injustice [121].

BIPOC and marginalized communities continue to encounter obstacles stemming from contaminated groundwater, which are exacerbated by the lack of resources and remediation assistance [122]. Addressing these disparities requires readily available data driven models to support the development of comprehensive policies, strengthened environmental regulations, and active community involvement. Community-engaged research is a crucial bridge between technical expertise and community empowerment in addressing environmental justice concerns for equitable outcomes [123].

This study investigates the intricate relationship between pesticide transport, groundwater vulnerability, and environmental justice in the context of climate change. To comprehensively assess the influence of pesticide transport on groundwater vulnerability, this study uses a geospatial analytical hierarchy overlay model. Pesticide transport, climate change, and related environmental justice issues are investigated. Furthermore, the research uses a robust analytical detection technique, specifically focusing on glyphosate and its metabolites concentrations, to validate and refine the developed spatial models. The study intends to enhance our understanding of the environmental complexities involved by contributing valuable insights for sustainable environmental implementation and management.

Methodology

Geospatial Analytical Hierarchy Overlay Model: Framework and Implementation

Case study locations

The Kentucky River Basin, spanning 7,000 square miles in northeastern Kentucky, is renowned for its rolling hills, forests, farmlands, and abundant waterways, with the central artery being the Kentucky River, which joins the Ohio River in Carrollton [124]. The basin is crucial for ecosystems, aquatic life, local communities, and the state's economy, providing water for drinking, industrial use, and agriculture [125]. Water samples were collected in two of its watersheds for the monitoring of glyphosate - Cane Run and Royal Spring, and Camden Creek (Table 4.1).

Cane Run and Royal Spring are groundwater networks in Kentucky's Inner Bluegrass Region (Figure 1) (Fayette and Scott counties), characterized by agricultural land use, urbanized headwaters, temperate climate, and moderately deep, well-drained soils supported by Middle Ordovician phosphatic limestone [19]. Two of the sampling points, near a printer company (PRINT) and a horse park (KYHP), were in highly dense urban areas whereas the other locations were in high agriculture and mixed forest areas. The Camden Creek watershed in Woodford County, Kentucky (Figure 4.1), is the drainage basin for a significant portion of the C. Oran Little farm, covering 4.2 square miles in total [126].

Table 4.1. Key demographic, economic, and environmental characteristics of the two counties and their respective watersheds [127]

Parameter	Fayette County, KY	Woodford County, KY
Racial / Ethnic Composition	White (70%), Black or African American (15%), Asian (4%), Hispanic (2.8%)	White (86%), Hispanic (4.7%), Black or African American (3.9%), Asian (2.7%)
Median Household Income	$61,526	$49,000
Characteristics of Sampling Points	High urbanization and mixed forest	High agriculture and mixed forest
Economic Significance	Reserve for distilleries, grist mills, horse farms, and crop irrigation	Agricultural and horse pastures

Watershed Vulnerability Indexing.

The delineation of watershed boundaries was conducted using ArcMap for Windows Software (Version 10) by the Environmental System Research Institute (Redlands, CA, USA), with data processed through ArcGIS software and transformed into GCS_WGS_1984 coordinates projection for compatibility. Relevant data were extracted from remote sensed digital elevation models (DEMs) and historical geological and topographical records, sourced from the Kentucky Geological Survey's KYGeoNet portal, housing federally mapped data pertaining to Kentucky.

Seven thematic layers [slope, precipitation, land use/land cover (LULC), population density, infiltration, drainage density, and lineament density] were employed to assess the hydrogeological and anthropogenic influences on glyphosate transport in karst watersheds, ensuring that the shortfalls of existing models were accounted for in the proposed model (Table 4.2) [128].

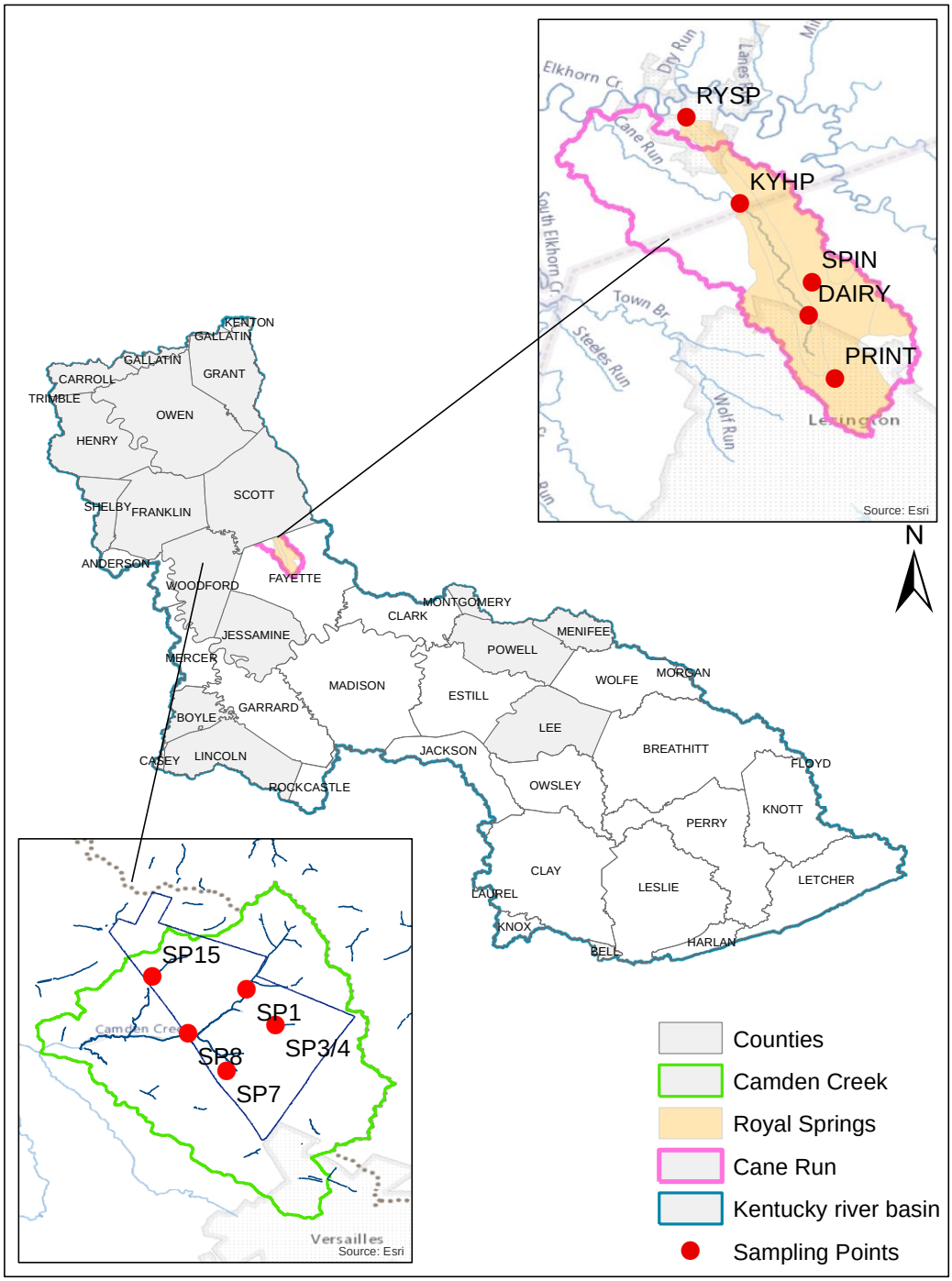


Figure 4.1. Map of Kentucky River Basin, Camden Creek and cane run and royal spring watersheds.

Table 4.2. Regional guide for the selection of thematic layers [128].

Thematic layers	**Overlay groundwater vulnerability index assessment tools**							
	Proposed Model	**DRASTIC** [72]	**GOD** [20]	**SINTACS** [129]	**EPIK [18]**	**PI** [130]	**ISIS** [131]	**AVI** [132]
Slope (degree)	✓	✓		✓				
Lineament Density (m^{-1})	✓	✓	✓	✓	✓		✓	✓
Drainage Density (m^{-1})	✓	✓	✓	✓	✓			
LULC	✓				✓	✓	✓	
Infiltration	✓	✓	✓	✓	✓	✓	✓	✓
Precipitation (mm)	✓	✓		✓				
Population Density (people/km^2)	✓							✓

Classes within each thematic layer were created and scored using the Saaty's scale ranging from 1-9 (Table 4.3) [78] . High Saaty's scores were assigned to higher classes within the thematic layers of slope, lineament, drainage density, infiltration, precipitation, and population density (Table 4.4 and Tables S4.1-S4.5). Table 4.4 demonstrates the pairwise comparison of the nine different slope classes. The higher classes in each thematic layer exert a greater influence on contamination vulnerability, attributed to the swift movement of pesticides [133]. The LULC classes were developed based on increasing glyphosate usage leading to the transport of pesticides and groundwater contamination (Table S4.6) [128].

Table 4.3. Rating score of Saaty's analytical hierarchical process (*IV = Intermediate values*) [78].

Score	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Degree of Preference	Equal	IV	Moderate	IV	Strong	IV	Very Strong	IV	Extreme

Table 4.4. Saaty's analytical hierarchy process pairwise comparison of slope classes.

Pairwise comparisons											
	Class Number	1	2	3	4	5	6	7	8	9	
Class Number	**Class Description (degree)**	**<0.57**	**0.58-1.1**	**1.2-1.8**	**1.9-2.5**	**2.6-3.2**	**3.3-4.2**	**4.3-5.6**	**5.7-8.4**	**8.5-13**	Weight
1	**<0.57**	1.00	0.50	0.33	0.25	0.20	0.17	0.14	0.13	0.11	2%
2	**0.58-1.1**	2.00	1.00	0.50	0.33	0.25	0.20	0.17	0.14	0.13	3%
3	**1.2-1.8**	3.00	2.00	1.00	0.50	0.33	0.25	0.20	0.17	0.14	4%
4	**1.9-2.5**	4.00	3.00	2.00	1.00	0.50	0.33	0.25	0.20	0.17	6%
5	**2.6-3.2**	5.00	4.00	3.00	2.00	1.00	0.50	0.33	0.25	0.20	8%
6	**3.3-4.2**	6.00	5.00	4.00	3.00	2.00	1.00	0.50	0.33	0.25	12%
7	**4.3-5.6**	7.00	6.00	5.00	4.00	3.00	2.00	1.00	0.50	0.33	16%
8	**5.7-8.4**	2.00	7.00	6.00	5.00	4.00	3.00	2.00	1.00	0.50	21%
9	**8.5-13**	1.00	8.00	7.00	6.00	5.00	4.00	3.00	2.00	1.00	29%
	CR Value	-0.19									

The seven thematic layers were grouped into three categories – 1) public health influence (population density), 2) pesticide runoff and groundwater interaction initiators (precipitation, infiltration and LULC) and 3) topographical influences (slope, drainage, and lineament densities). Population density was scored highest in the thematic layer comparison matrix to provide a comprehensive understanding of groundwater vulnerability by capturing the multifaceted interactions between human activities, land use, and potential contamination risks (Table S4.7). Population density is a crucial parameter for local stakeholders and its inclusion considers their concerns and priorities, thereby validating goals and characteristics of the study area [134]. Prioritizing pesticide runoff and groundwater interaction initiators over topographical influences enhanced the assessment's relevance by focusing on direct contamination pathways [74].

Precipitation was then scored higher than infiltration and LULC due to its direct impact on recharge and connection to the hydrological cycle which heavily influences contaminant transport in groundwater connectivity [75]. Infiltration received a higher score than LULC owing to its intermediate role in initiating and transporting pesticides in groundwater vulnerability, particularly within a well-developed karst basin [21]. This prioritization ensures a more precise depiction of the factors driven by runoff that influence groundwater vulnerability. In evaluating topographical influences, the highest score was assigned to drainage density, followed by lineament density, and then slope. Drainage density increases connectivity between groundwater and surface water systems, enhancing the potential for contaminants to reach groundwater through surface runoff [74]. Lineament density influences groundwater flow patterns and contributes to contaminants' movement

whereas slope influences runoff speed and surface water interaction [135]. Drainage density, lineament density, and slope are all measurable parameters, enhancing the quantitative aspect of the vulnerability assessment. The groundwater vulnerability index (GVI) was calculated by summing each thematic layer's AHP weight and Saaty's score of each thematic layer weighted values, as illustrated in Equation 4.1. This process facilitated the segmentation of the entire study area into distinct GVI zones.

Groundwater Vulnerability Index (GVI) =

$$S_w S_c + LU_w LU_c + L_w L_c + I_w I_c + D_w D_c + R_w R_c + P_w P_c \quad \textbf{(Equation 4.1)}$$

where land slope = S (degrees), land cover/use = LU, lineament (meter $^{-1}$) = L (meters), infiltration = I, precipitation = R (millimeters), population density = P (people/kilometer^2), and drainage density to groundwater = D (meter^1). AHP weight of each layer = *'w'*, Saaty's score of individual thematic layer = *'c'*

The model validation process utilized map removal sensitivity analysis to assess the vulnerability map's robustness by systematically removing thematic layers and comparing original and modified models to determine each layer's impact on accuracy, identifying crucial maps for precise spatial mapping. JMP statistical software was utilized for spatial variance and robustness evaluations using Dunnett's test, alongside geospatial correlation analysis to understand groundwater vulnerability's interaction with environmental justice, guiding environmental policy decisions. Spatial analysis investigated the overlap between highly vulnerable areas and federally designated opportunity zones [136], enhancing

understanding for management decisions. The uncertainty of the GVI was quantified through the calculation of the consistency ratio (CR) of the AHP matrix (Equation 4.2).

$$CR = (\lambda max - n) / [(n - 1) * RI] \qquad \textbf{(Equation 4.2)}$$

where λmax = maximum eigenvalue of pairwise comparison matrix, n = number of criteria being compared, and Random Index = RI (pre-determined for 7 thematic layers = 1.3) [28]. If the CR is less than or equal to 0.1, the judgments are considered consistent and reliable.

Analytical Detection Techniques for Glyphosate and Metabolites Concentrations

The Environmental Protection Agency (EPA) surface water sampling protocol and Section 8 of EPA Method 547 for glyphosate assessment in drinking water were followed in the collection of surface and groundwater grab samples [137, 138]. A YSI multiparameter meter was used to measure temperature, conductivity, dissolved oxygen, salinity, total dissolved solids, chloride, and ammonia levels at each sampling point. The study used a pre-column derivatization protocol developed by Martin et. al [14]. The sample was transferred into an amber glass bottle, supplemented with 800 µL of isotope internal standard solution. 9-fluorenylmethyloxycarbonyl chloride (FMOC-Cl), ethylenediaminetetraacetic acid (EDTA) and borate solutions were then added. The samples were incubated in a water bath at 40°C in darkness for 4 hours, then phosphoric acid solution was added and stored at 4°C. The study used an Agilent Series 1290 LC coupled with an Agilent 6470A triple quadrupole mass spectrometer. The drying gas flow rate was set at 5 L/min at 300°C, with a nebulizer pressure of 45 psi and a fragmentor voltage of 110 V. Agilent Masshunter Qualitative and Quantitative

software was used for processing the chromatographic results. A Phenomenex Gemini NX-C18 column was used, with each injection containing 20µL matrix/sample and maintaining a column compartment temperature of 30°C.

Community-Centered Approach in Research Design

Embracing a community-centered approach in the research design ensured the active engagement and collaboration of various stakeholders to comprehensively address environmental concerns within the community. By leveraging partnerships with key entities such as the Georgetown water treatment plant (RYSP), University of Kentucky research farms (DAIRY and SPIN), and the Cooperative Extension Service Program (all sites in Camden creek), a holistic understanding of the local landscape, including areas of need, contaminants of concern, and prevalent societal practices, was achieved. This collaborative endeavor not only facilitated the identification of pressing environmental issues but also allowed for the exploration of underlying socio-economic factors that influence environmental health. Through ongoing dialogue and consultation with community leaders and stakeholders, shared understanding of priorities emerged, guiding the direction of the research process.

Informed by insights gleaned from community engagement initiatives, sampling efforts were strategically directed towards sites of interests formed by community leaders. This targeted approach ensured that research activities were not only scientifically rigorous but also directly relevant to the lived experiences and concerns of residents. By centering the research design around community needs and perspectives, the study sought to foster a

sense of ownership and empowerment among community members, positioning them as active participants in the research process rather than passive subjects.

Furthermore, the collaborative nature of the research endeavor facilitated knowledge exchange and capacity-building initiatives within the community. A partnership with a local high school, STEAM Academy Lexington KY, not only facilitated data collection but also ensured effective dissemination of knowledge within the community. By building local capacity and fostering collaborative relationships, the research aimed to lay the foundation for sustainable, community-driven solutions to environmental challenges.

Results and Discussion

Spatial Mapping of Pesticide Transport and Groundwater Vulnerability

A significant portion of the Kentucky River Basin lies within the <1.8° slope category, predominantly in the northern regions, where the slope is gentlest, and the topographic elevation is lowest. Higher slope classes are primarily found in the southeastern areas of the basin (Figure 4.2a). Effective pesticide application promotes agricultural productivity; however, steep slopes can alter subsurface water movement, preventing intended use on application site.. This, in turn, jeopardizes food production and security [139]. The lineament densities across the basin range from 0.1 to 19 m^{-1} (Figure 4.2b). The study reveals a decrease in lineament density towards the southwest, while higher density is observed in the northeastern regions, forming an elongated shape. Particularly in regions with a high density of lineaments, lineament density increases the connectedness of subsurface flow routes, enabling pesticides to reach groundwater more quickly and possibly increasing contamination risk [73]. Most of the study area exhibits low to moderate drainage densities (Figure 4.2c). Drainage densities <2.4 m^{-1} encompass 66% of the basin area, while densities ranging from 2.5 to 7.1 m^{-1} cover 9%. Additionally, densities between 7.2 and 11 m^{-1} occupy 11% of the area, densities of 12-16 m^{-1} cover 8%, and densities exceeding 17 m^{-1} account for 6% of the basin.

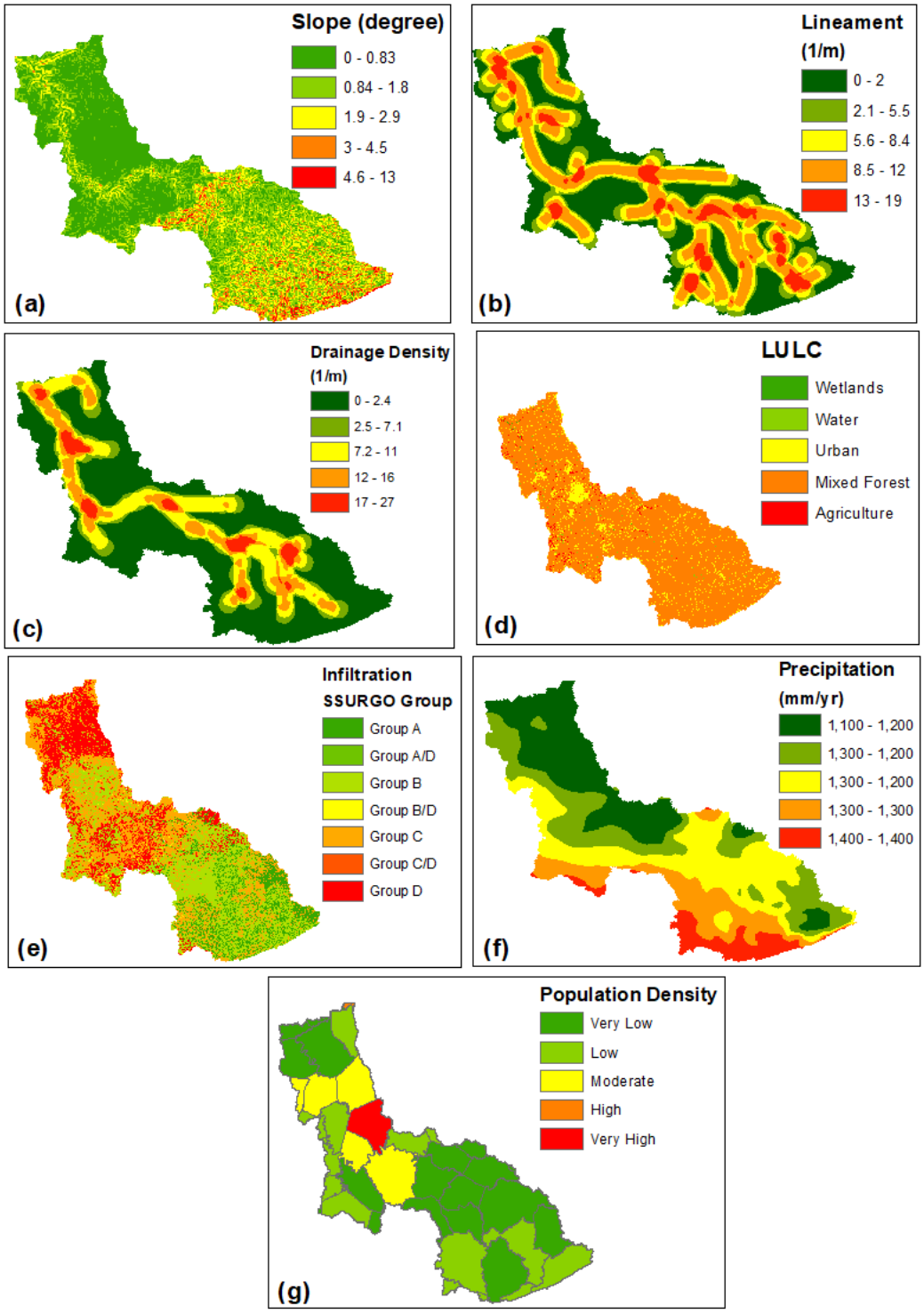


Figure 4.2. Map of thematic layers including (a) slope, (b) lineament, (c) drainage density, (d) LULC, (e) infiltration, (f) precipitation and (g) population density.

The geological and climatic conditions in the watersheds play a significant role in determining channel length which influences drainage density. This is primarily due to the presence of low relief vegetation cover and permeable karst subsurface materials [140]. Urban areas, mixed forests, and agricultural are the main LULC types in the Kentucky basin (Figure 4.2d). In contrast, water bodies and wetlands constitute a minor proportion of the overall LULC type. Factors like fertilizer use, forest dynamics, urbanization, and water bodies and wetlands can affect groundwater recharge and ecological balance [1]. Monitoring and managing these areas are crucial for sustainable water resources.

Most of the northern and central sections of the basin are characterized by slow infiltration (Group C, C/D and D) (Figure 4.2e). These topographies have poor water-absorbing capacities and restricted drainage, which leads to increased runoff (Table S4.8). The presence of karst plains impacts pesticide drift and groundwater contamination. High infiltration levels in watersheds suggest potential for leaching and runoff, as water and pesticides easily move through soil and aquifer connections [73, 133]. The average annual rainfall observed within the basin showed highest levels in the southern part whilst the northern section experienced the lowest amount of precipitation (Figure 4.2f). Groundwater sources are particularly vulnerable to glyphosate contamination due to precipitation-induced runoff, erosion, and leaching [140].

Figure 4.2g highlights the low population densities of 90% of the total Kentucky River Basin area. Fayette county has the highest population density, influenced by historical settlement patterns, economic opportunities, and geographic features [141]. This leads to

increased pesticide use, potential exposure, and glyphosate in groundwater due to urban and agricultural activities and raises concerns about water safety and sustainable management, emphasizing the importance of clean water access for human wellbeing [6].

The GVI classification (Figure 4.3) reveals varying risk levels across the study area. The outcomes of the groundwater vulnerability assessment reveal that 18% of the area falls under the very low-risk classification, 23% as low-risk, 27% as prone to risk, and 20% and 12% as high-risk and very high-risk areas, respectively. The CR value for all pairwise matrices were <0.1 showing that the judgments are highly acceptable [25]. The hotspots of risk are predominantly concentrated in the mid-eastern and southeastern portions of the watershed. Fayette county, being the largest in the county population density and rich in biodiversity, was identified as highly susceptible to pesticide exposure [124, 125, 142]. The overlap of biodiversity hotspots, availability of water storage resources, and areas of high-risk pesticide further highlights the urgency of addressing pesticide transport. Implementation of precision agriculture techniques and buffer zones around sensitive areas are crucial steps to mitigate glyphosate drift and protect the region's ecological and social sustainability [143]. Areas with karst topography exhibited the highest vulnerability due to the high infiltration and direct contact with routes for the runoff in surface water interaction with groundwater [144-146].

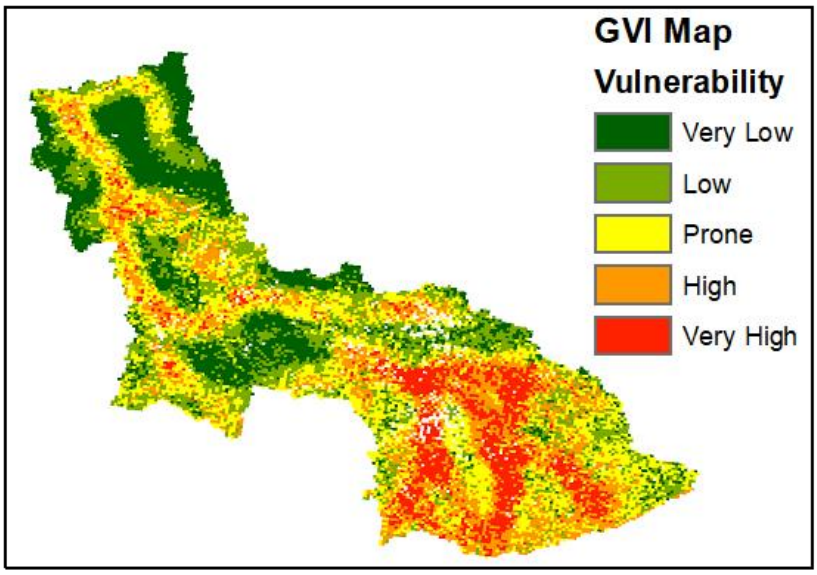


Figure 4.3. GVI map for Kentucky River Basin.

Significant changes in classification of risk areas were seen when any of the thematic layers were removed individually ($p<0.05$). The results show that these thematic layers affect the movement of glyphosate within the watershed and have significant influence on the vulnerability of groundwater sources. Glyphosate groundwater contamination posed the biggest threat to individuals in the high populated regions of the watershed, indicating the importance of high scoring and weights associated with the influence of population density. Glyphosate's environmental and health risks are linked to the interdependence of natural and human factors, highlighting the need for optimal management practices to achieve sustainable usage strategies [6]. Public awareness and education about glyphosate hazards and proper handling and disposal methods are also crucial. Implementing climate-resilient practices and sustainable urban design can reduce pesticide usage in agriculture and urban areas during vulnerable times of heavy precipitation [147, 148].

Although high glyphosate usage is usually attributed to high agricultural areas with large-scale intensive farming, high-vulnerability zones were observed in economically non-developed areas with little or no agriculture. Underscoring the occurrence and influence pesticide transport from application regions. Sustainable agriculture, forestry, and water resource management are crucial for biodiversity, climate change, and water protection. Prioritizing responsible consumption, improving laws, and developing collaborations are essential [7]. Continuous monitoring, research, and cooperation are needed for informed decisions, policies, and long-term practices [3, 14].

Validation of Models through Glyphosate Concentration Analysis

The water quality analysis (Table S4.9) revealed that dissolved oxygen levels across all samples exceeded the EPA's minimum requirement for warm water aquatic life (>5 mg/L) [149], likely influenced by microbial interactions leading to increased glyphosate photodegradation [150]. While most sites met EPA freshwater salinity guidelines, DAIRY and KYHP exhibited elevated salinity, indicating high water hardness and the presence of various metal cations [151, 152]. High water pH inhibited glyphosate uptake, with pH levels within the 6–9 range [153]. Nitrate and ammonia levels generally complied with EPA standards, except for SP7 in the Camden Creek watershed, where ammonia exceeded EPA limits [141]. However, total nitrogen at SP7 remained within WHO guidelines for drinking water [153]. Elevated nitrate-nitrogen concentrations increase the likelihood of detecting mobile pesticides [154], but glufosinate concentrations in sampled watersheds remained below WHO's maximum residue limit [3], suggesting safe exposure levels. There was no discernible

pattern of glufosinate concentration fluctuations correlated with rain events, indicating minimal transport from fields to surface water (Figure S4.1 and Figure S4.2).

Glyphosate was detected in both Camden Creek and Cane Run and Royal Spring watersheds with average concentration of 0.40 ± 0.023 μg/L and 0.55 ± 0.11 μg/L respectively. The highest concentrations were found in the highly intensive agriculture sampled sites (DAIRY and SPIN) for the Cane Run and Royal Spring watershed. The average glyphosate concentration detected was higher than the concentration of AMPA found. The relatively high concentrations of glyphosate and AMPA in the downstream sampled outlet sites (PRINT and SP 8) does indicate a potential transfer of these pesticides along the watershed. The inverse proportional trend in glyphosate and AMPA concentrations validates glyphosate as the primary source of AMPA (Figures S4.1 and S4.2) [3, 14]. The ubiquitous levels of glyphosate and AMPA found in urban sampling locations (PRINT and KYHP) indicate that glyphosate applications used for non-agriculture purposes may have a significant role in the overall pollution of surface waters. This non-point glyphosate contamination sources includes weed control along highways, railways, residential areas, parks, and golf courses [1]. Empirical data from several researchers supports this significant contribution of urban activities [1, 22]. Although the detected levels of glyphosate adhere to the EPA's environmental quality standards, the extensive use of glyphosate in urban settings may pose significant public health risks [150]. This leads to elevated concentrations in surface water, underscoring the necessity for ongoing monitoring and the establishment of sustainable usage practices. Such initiatives are vital for mitigating glyphosate concentrations in areas at heightened risk [3, 118, 122, 143]

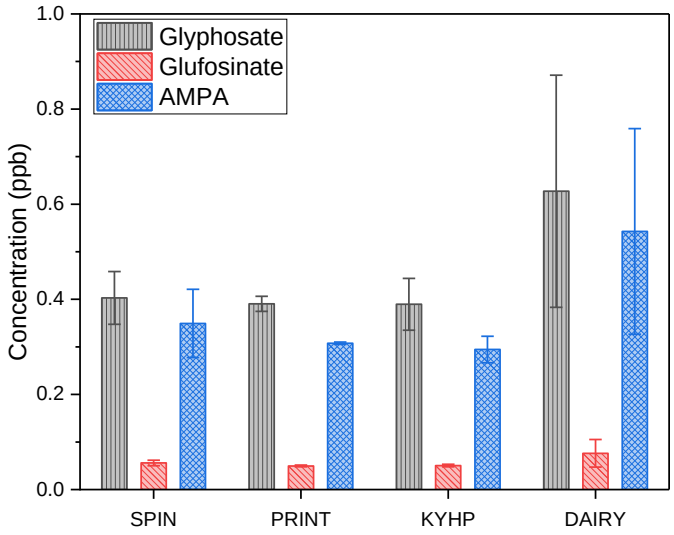


Figure 4.4. Averaged detected concentration of glyphosate, glufosinate and AMPA from January 2022 to June 2022 in the Cane Run and Royal Spring watershed. *(large standard deviation in AMPA and glyphosate concentrations for DAIRY due to significant detection variations).*

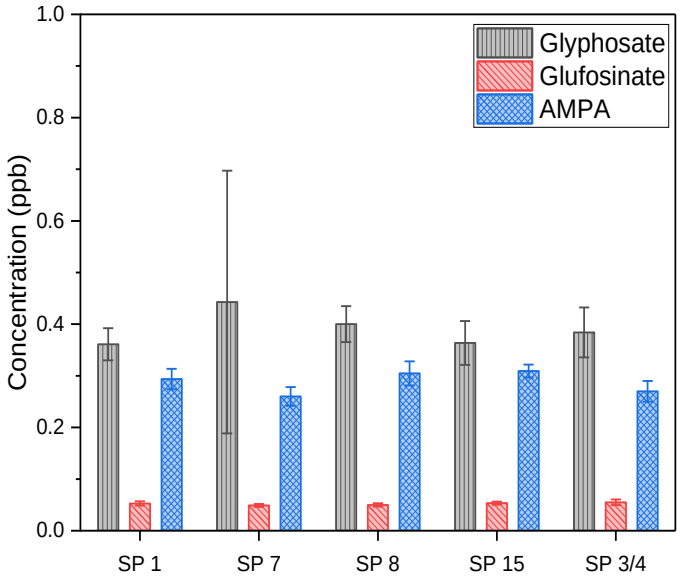


Figure 4.5. Averaged detected concentration of glyphosate, glufosinate and AMPA from January 2022 to June 2022 in the Camden creek watershed. *(large standard deviation in glyphosate concentration for SP 7 due to significant detection variations).*

Identification of Environmental Justice Hotspots

A significant section of either prone, high, or very high groundwater vulnerability mapping were found in low population density areas, south of the study area ($p<0.05$) (Figure 4.6a). However, 70% of the most populated county in the basin, Fayette County, was observed to be in the prone, high, or very high GVI classification. This indicates a substantial amount of the population in the basin are potentially exposed to glyphosate contamination given that the Bluegrass area relies on aquifer reserves for public drinking water [142]. Surface water entering the groundwater reserves and aquifers via the predominant karst systems characterizing the basin is most likely the source of the pesticides affecting the human health, environmental quality and socioeconomic development of the area [118].

Despite the usage of glyphosate in the northern section of the watershed, only 22% of the area fall in either prone, high, or very high groundwater vulnerability compared to the 86% of the southern sector which accounts for 21% of the total glyphosate usage (Figure 4.6b). The behavior of pesticides in urban catchments is poorly known and documented [155], however the low GVI in application and high usage sites indicate the transport of glyphosate to unintended locations. The transport of glyphosate evidenced by the spatial discrepancy raises concerns about the potential environmental impact of this pesticide movement to the ecological and water quality implications of the watershed [22]. The relationship between pesticide exposure and marginalized communities is intricate. The glyphosate contamination of groundwater sources in the basin disproportionately impacted low-income communities (Figures 4.6c and 4.6d). All federally designated opportunity zones fell in either prone, high, or very high groundwater vulnerability (Figure 4.6c). This

intersection underscores the need for careful planning, community engagement, and sustainable development practices to avoid exacerbating environmental disparities and ensure that economic opportunities are distributed equitably across all communities [121].

Figure 4.6e highlights the relationship between the distribution of BIPOC communities and GVI mapping. A comparison with Figures 4.6c and 4.6d brings forth the intersectionality of the low income and BIPOC areas. Across the Kentucky River Basin, BIPOC communities are spread out, particularly concentrated in the densely populated northwest region. Notably, in the southern part, a cluster of BIPOC communities are found in areas with high populations with low-income status. These regions coincide with hotspots of vulnerability to groundwater contamination. The southern border of the Bluegrass area exhibits high GVI classification, aligning with the predominant residence of BIPOC communities in that area.

The observed correlations between the GVI mapping and environmental justice indicators are the result of historical injustices and contemporary regulatory practices that perpetuate disparities in pesticide exposure and harm [6]. This is critical for comprehending how a variety of factors, including proximity to agricultural activities, occupational pesticide contact, and pollution concentration in marginalized areas, influence these disparities in addition to race and income. This link between BIPOC, low-income communities, and high GVI underscores the persistent environmental injustices these groups endure. This reinforces the urgency for comprehensive solutions addressing both the immediate and enduring effects of pesticide pollution on these communities [6, 118].

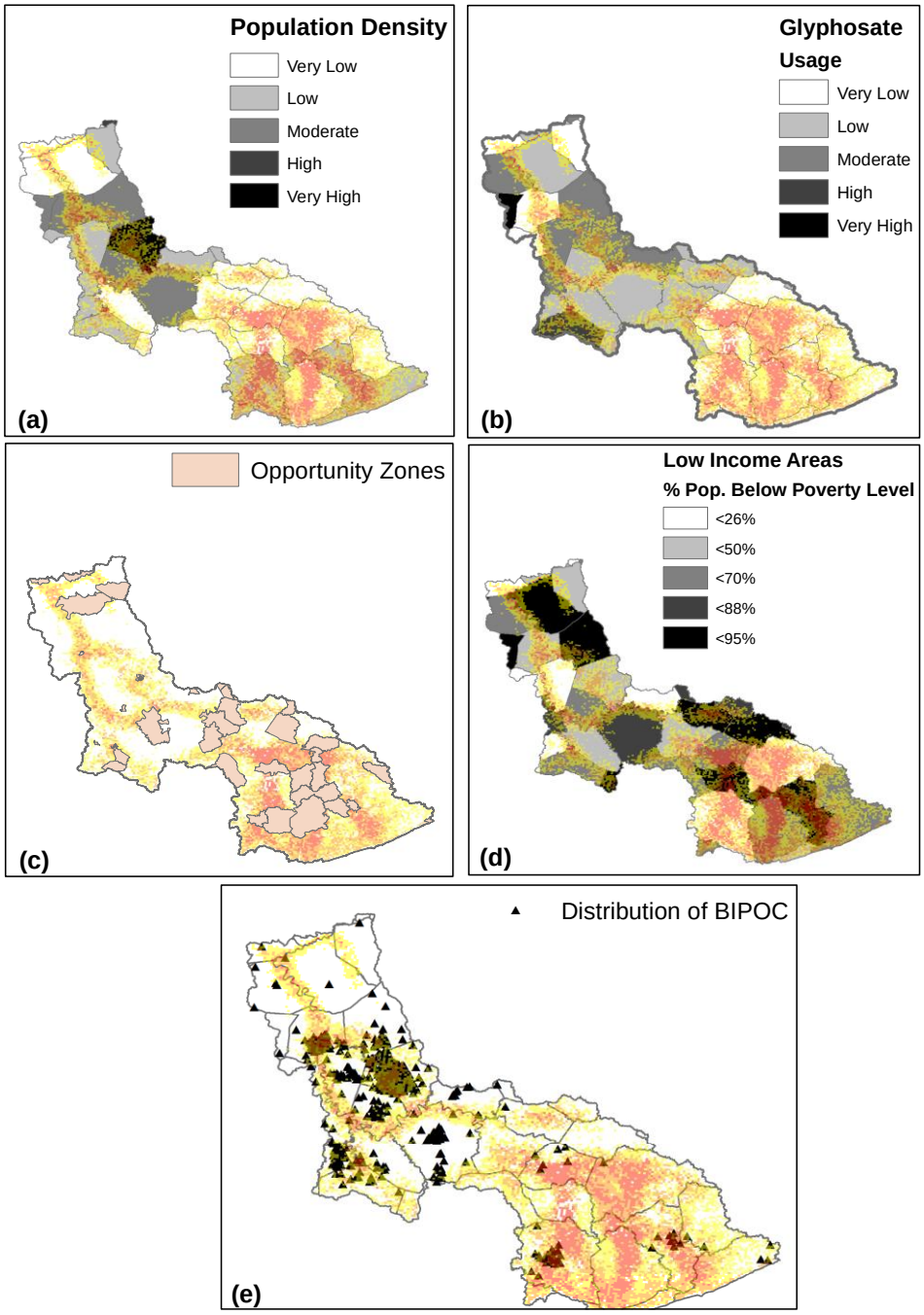


Figure 4.6. Overlay map of GVI high vulnerability zones on (a) population density, (b) glyphosate usage map, (c) federal opportunity zones, (d) low-income areas map and (e) the distribution of BIPOC communities (GVI map: yellow = prone, orange = high, and red = very high).

Conclusion

Future community-engaged environmental engineering projects should prioritize monitoring vulnerable areas for glyphosate contamination, employing geospatial technologies and detection techniques, while involving local communities and stakeholders in decision-making to ensure environmental justice. Advocating for policy reforms and considering climate change's impact on pesticide transport pathways are crucial steps for equitable and sustainable solutions. The research utilizes a multi-disciplinary approach, including geospatial analytical hierarchy overlay models and robust detection techniques, revealing that 59% of the Kentucky basin is moderately or highly vulnerable to groundwater contamination by glyphosate runoff, with most vulnerable areas in the central and southeastern sections. Detected glyphosate and aminomethylphosphonic acid (AMPA) concentrations were elevated in areas with intensive agriculture, particularly within the Camden Creek, Cane Run, and Royal Spring watersheds, suggesting potential pesticide transfer downstream. Urban areas also exhibited significant glyphosate and AMPA presence, likely from non-agricultural uses. Additionally, the study underscored the unequal distribution of environmental impacts on public health and environmental risks of pesticides, particularly in areas like the Kentucky River Basin, highlighting disparities associated with population density, Black, Indigenous, and people of color communities (BIPOC), and low-income areas. These findings emphasize the importance of addressing environmental justice issues and ensuring marginalized communities have equitable access to a clean and safe environment, informing more inclusive environmental policies and practices that consider the impact of climate change on pesticide transport.

CHAPTER 5

SUSTAINABLE ENVIRONMENTAL MANAGEMENT VIA SPATIAL MODELING AND MAPPING OF GLYPHOSATE VULNERABILITY ZONES IN BELIZEAN WATERSHEDS.

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This chapter is under review at a peer-reviewed journal. This chapter addresses research questions 2) What are the spatiotemporal dynamics governing the transport and distribution of glyphosate, glufosinate, and AMPA within watersheds, and how can remote sensing technologies and groundwater interconnectivity models inform strategies for mitigating environmental impacts? and 3) What socio-economic and environmental justice factors influence the interplay between agricultural practices, glyphosate usage, and societal equity considerations, and how can this understanding inform the development of sustainable management practices tailored to diverse rural and urban landscapes?

Abstract

The unintended transport of pesticides from application sites poses significant threats to environmental sustainability, biodiversity, and public health potential adverse effects on water quality contradicting UN Sustainable Development Goals. To address these concerns,

this study aims to develop a geographic information system (GIS) and remote sensing (RS) - based evaluation tool to assess potential groundwater contamination by glyphosate in Belize, aligning with sustainable development objectives for water resource management. The study focuses on 39 watersheds in Belize and uses a geospatial and geostatistical analytical overlay technique to identify high-risk locations for glyphosate vulnerability zones. Results from the study finds that 24% and 14% of the watersheds in Belize are at a high and very high risk to the occurrence of glyphosate respectively ($p<0.05$). Severe glyphosate runoff in agricultural areas with significant karst and high population density was observed ($p<0.05$) and highlights the vulnerability of drinking water sources. Misuse of pesticides in water sources management may have considerable long-term implications for drinking water supply, which would obstruct efforts to achieve SDGs 3, 6, 11, and 12 by 2030. This research highlights the importance of sustainable pesticide drift modeling, enhances watershed management practices, and facilitates effective monitoring efforts to ensure environmental sustainability, biodiversity conservation, and public health.

Keywords: pesticide transport, sustainable agriculture, environmental regulation, geospatial analysis, groundwater vulnerability

Introduction

The use of pesticides threatens the United Nations (UN) Sustainable Development Goals (SDGs), prompting a focus on reducing vulnerability to non-target species, the environment, and public health [35, 156]. Despite the efficacy of glyphosate-based herbicides in bolstering agricultural productivity and ensuring food security (SDG 2), significant threats to environmental sustainability (SDG 12), biodiversity (SDG 15), and human health (SDG 3) may arise due to the unintended drift from application areas [39, 157]. The introduction of glyphosate in the Belizean market has significantly increased agricultural productivity and development, boosting the global economy, and its use has surged over the past two decades [158, 159]. Concerns and controversy over the bioaccumulation, maximum contaminant level (MCL) and toxicity of glyphosate and its metabolite, aminomethylphosphonic acid (AMPA), has prompted regulatory unease leading to recent classification as a priority pesticide of concern due to potential water contamination in Belize [144, 160, 161].

The persistence of pesticide residues in water systems contributes to pollution and ecosystem degradation, exacerbating challenges related to water quality and access to clean water sources, as per SDG 6 [162]. The quantification of pesticide export within watersheds is challenging due to the use of different ingredients, methods, and environments, making it challenging to gauge the scope of the problem [163]. Additionally, the complex influences and hotspots of long-term pesticide use remain uncertain. Although spatial models have been used to forecast the transport and fate of chemicals, there are still obstacles such as pesticide legacy, mixtures, and varying receptors and endpoints [164, 165]. A

spatiotemporal analysis of pesticide risk vulnerability in Belize has become necessary to protect groundwater to achieve the SDG target of clean water and sanitation.

Quantifying the concentrations that occur in various habitats and lowering potential pesticide ecological hazards are crucial for properly comprehending the consequences of pesticide dangers under excessive usage. Tools for geographic information systems (GIS) and remote sensing (RS) can lead to new directions in the study of water resources [23]. Analysis of remote sensing data with necessary ground truth verifications helps in generating the baseline information for groundwater targeting. Identification of groundwater contamination by pesticide drift using remote sensing data is based on indirect analysis of directly observable terrain features like geology, geomorphology, and their hydrologic characteristics [24-26]. For effective management of water resources, GIS and RS can be used in resource evaluation and hydro-geomorphological mapping [166]. The assessment of pesticide risks to both the environment and human health is a critical component of achieving sustainability goals in pesticide management.

The aim of this study is to develop a customized analytical hierarchy process (AHP) evaluation tool consisting of GIS and RS techniques to delineate and analyze potential zones for groundwater contamination resulting from pesticide transport. This objective entails spatial mapping and assessing the potential for groundwater contamination by glyphosate, employing integrated approaches that align with sustainable development, planning, and optimal utilization of groundwater for both irrigation and drinking purposes. Layers such as geology, slope, soil type, landforms, lineament density, drainage density, rainfall, land

use/landcover, and pesticide drift parameters were all considered. The findings of the study were corroborated with the vegetation cover of the agroecosystem within the selected areas.

Methodology

Study site.

Belize is known for its biodiversity; agriculture and tourism are the country's two most important economic sectors, accounting for around 22% of total land utilization in the watersheds in 2016 [3, 114, 144]. The middle reaches of the watershed are the primary areas for agricultural activities, and the majority of its groundwater reservoirs are replenished by the Belize River. [167]. Pesticide spatial modeling is critical for detecting significant contamination in watersheds that support the country's agriculture industry [114]. The study encompassed all 39 watersheds in Belize along with the entire mainland (Figure 5.1), selected for analysis due to glyphosate application sites, proximity, and ecosystem services.

The sites, characterized by diverse ecosystems, collectively spans 22,970 square kilometers and serves as vital sources of drinking water for rural and urban regions nationwide [144]. The Belize River connects all of Belize's watersheds. The Belize River is a 180-mile-long river that begins at San Ignacio, Belize, at the confluence of the Mopan and Macal Rivers and runs into the Caribbean Sea near Belize City, passing through several Belizean districts such as Cayo, Stann Creek, and Toledo [160]. Glyphosate is the most widely used herbicide in Belize, 31% of total pesticide imports, for many crops, such as sugar cane, corn, grain, beans, citrus, and banana [160]. The watersheds in Belize, characterized by extensive agricultural operations and potential glyphosate influence on groundwater, are an

ideal study site. They offer a representative model of areas heavily affected by agriculture and glyphosate use, providing valuable insights into groundwater contamination risks for similarly intensified agricultural regions.



Figure 5.1: Map of Belize.

Data Preparation and Evaluation for Spatial Mapping

In this study, seven thematic layers were created by integrating RS data, specifically a digital elevation model (DEM), along with historically recorded information like geology and topography (Figure 5.2). Preparation of the thematic layers involved extraction and digitization of relevant description from multiple data sources. The DEM data utilized in this research was sourced from the Earth Observation for Sustainable Development project in

June 2016, which resulted from a collaborative effort between the World Bank (WBG) and the European Space Agency (ESA). The spatial resolution of the DEM was set at 30 meters. To assess the soil quality within Belize's watersheds, the Food and Agriculture Organization of the United Nations (FAO) digital soil map was employed. Additionally, land use data from the FAO repository under the Convention to Combat Desertification (UNCCD) was incorporated. These thematic layers were designed to emphasize the hydrogeological and anthropogenic factors affecting the transport of glyphosate.

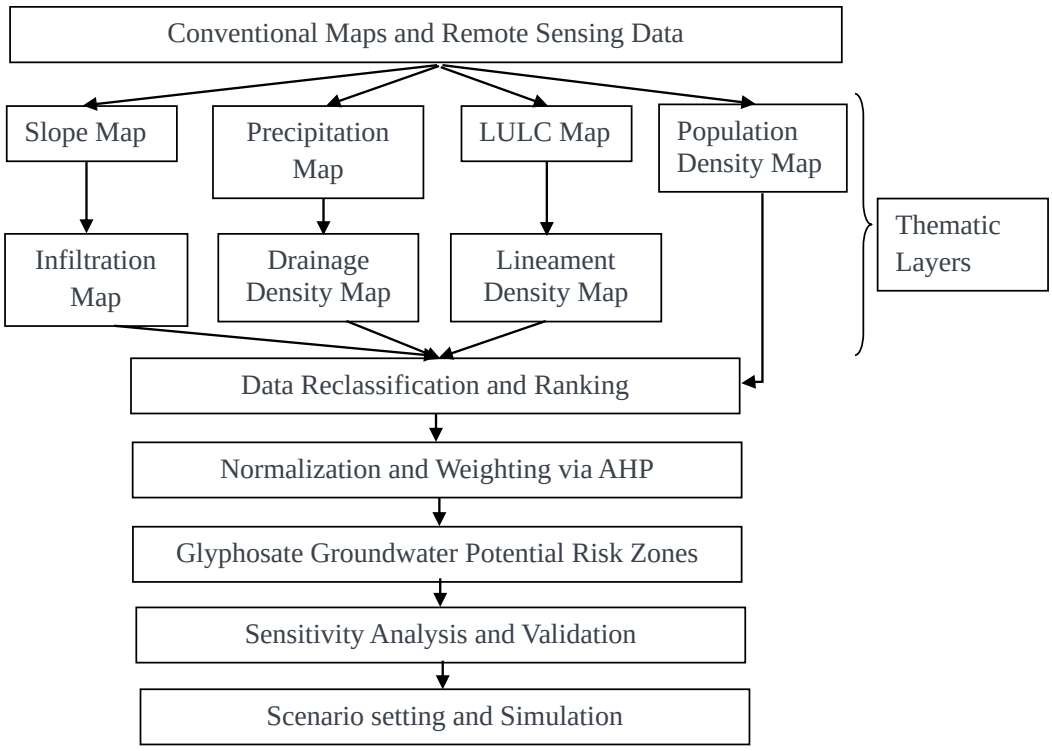


Figure 5.2. The flow diagram represents methodology for identification of glyphosate vulnerability zones.

The seven thematic layers consist of slope, precipitation, Land Use/Land Cover (LULC), population density, infiltration, drainage density, and lineament density. To establish the boundaries of different watersheds, a delineation process was conducted using ArcMap for Windows Software (Version 10), developed by Environmental System Research Institute Inc. located in Redlands, CA, USA. All digital data employed in this study underwent processing through ArcGIS software. To ensure compatibility, the map layers were transformed into WGS 1984 coordinates projection, aligning them with the FAO Soil Map coordinates projection. The software system's unit meter was used to define the distance scale. For rainfall data in the watersheds, information from the primary meteorological station under the Belize Metrological service was utilized.

AHP Computation and Modeling

The thematic layers were organized into classes and assessed using Saaty's scale, which spans from 1 to 9 (Table 5.1) [78]. Superior classes within each thematic layer, such as slope, lineament, drainage density, infiltration, precipitation, and population density were allocated higher scores. Table 5.2 illustrates the pairwise evaluation of the nine distinct slope classes. Greater Saaty's scores were assigned to the top classes in each thematic layer, indicating their heightened impact on contamination vulnerability, primarily due to the rapid dispersion of pesticides [133].

Table 5.1. Rating score of Saaty's analytical hierarchical process (IV = Intermediate values) [78].

Score	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Degree of Preference	Equal	IV	Moderate	IV	Strong	IV	Very Strong	IV	Extreme

Table 5.2. Saaty's analytical hierarchy process pairwise comparison of slope classes.

Pairwise comparisons											
	Class Number	1	2	3	4	5	6	7	8	9	
Class Number	**Class Description (degree)**	**<5.7**	**5.7 - 13**	**14 – 21**	**22 - 30**	**31 - 39**	**40 - 51**	**52 - 64**	**65 - 84**	**85 - 210**	Weight
1	**<5.7**	1	0.5	0.33	0.25	0.2	0.17	0.14	0.13	0.11	2%
2	**5.7 - 13**	2	1	0.5	0.33	0.25	0.2	0.17	0.14	0.13	3%
3	**14 – 21**	3	2	1	0.5	0.33	0.25	0.2	0.17	0.14	4%
4	**22 - 30**	4	3	2	1	0.5	0.33	0.25	0.2	0.17	6%
5	**31 - 39**	5	4	3	2	1	0.5	0.33	0.25	0.2	8%
6	**40 - 51**	6	5	4	3	2	1	0.5	0.33	0.25	12%
7	**52 - 64**	7	6	5	4	3	2	1	0.5	0.33	16%
8	**65 - 84**	8	7	6	5	4	3	2	1	0.5	21%
9	**85 - 210**	9	8	7	6	5	4	3	2	1	29%
	CR Value	-0.19									

The assessment prioritized factors contributing to pesticide runoff and groundwater interaction, emphasizing direct contamination pathways over topographical factors (Table 5.3). Population density emerged as the highest scored parameter in the thematic layer comparison, offering a holistic view of groundwater vulnerability by accounting for human activities, land use, and contamination risks. This inclusion acknowledges local stakeholders' concerns, validating the study area's goals and characteristics [74, 134]. Precipitation was prioritized over infiltration and land use/land cover due to its direct influence on recharge and its integral role in the hydrological cycle, which significantly impacts contaminant transport in groundwater. Infiltration received a higher score than land use/land cover due to its intermediate role in initiating and transporting pesticides in groundwater vulnerability, especially in well-developed karst basins. This prioritization ensures a more accurate representation of runoff-driven factors influencing groundwater vulnerability. In terms of topographical influences, drainage density received the highest score, followed by lineament density, and then slope [21, 75]. Drainage density enhances connectivity between groundwater and surface water systems, increasing the potential for contaminants to reach groundwater via surface runoff. Lineament density affects groundwater flow patterns and contributes to contaminant movement, while slope influences runoff speed and surface water interaction [74, 135].

Table 5.3. Saaty's Relative importance matrix for the thematic layers via AHP.

Thematic Layers	**Slope (degree)**	**Lineament Density(m^{-1})**	**Drainage Density(m^{-1})**	**LULC**	**Infiltration**	**Precipitation (mm)**	**Population Density (people/km^2)**	**AHP weight (%)**
	Scores							
Slope (degree)	1	1/2	1/3	1/4	1/5	1/6	1/7	**3%**
Lineament Density (m^{-1})	2	1	1/2	1/3	1/4	1/5	1/6	**5%**
Drainage Density (m^{-1})	3	2	1	1/2	1/3	1/4	1/5	**7%**
LULC	4	3	2	1	1/2	1/3	1/4	**11%**
Infiltration	5	4	3	2	1	1/2	1/3	**16%**
Precipitation (mm)	6	5	4	3	2	1	1/2	**24%**
Population Density (people/km^2)	7	6	5	4	3	2	1	**35%**

The groundwater vulnerability index (GVI) was calculated by summing each thematic layer's AHP weight and Saaty's score of each thematic layer weighted values, as illustrated in Equation 5.1. This process facilitated the segmentation of the entire study area into distinct GVI zones.

Groundwater Vulnerability Index (GVI) =

$$S_w S_c + LU_w LU_c + L_w L_c + I_w I_c + D_w D_c + R_w R_c + P_w P_c \quad \textbf{(Equation 5.1)}$$

where land slope = S (degrees), land cover/use = LU, lineament (meter $^{-1}$) = L (meters), infiltration = I, precipitation = R (millimeters), population density = P (people/kilometer^2), and drainage density to groundwater = D (meter^1). AHP weight of each layer = *'w'*, Saaty's score of individual thematic layer = *'c'*

Spatial Model Validation

The study employed spatial model validation to assess the significance of each map in enhancing the model's robustness for evaluating potential vulnerability zones of glyphosate in groundwater. This validation process involved conducting sensitivity analysis using JMP statistical software (Cary, NC), focusing on the simulated GVI maps. Each map was systematically removed from the model, and the model was reevaluated without the excluded map to assess its contribution to the model's accuracy. Maps that did not significantly contribute to the model's robustness were omitted, aiding in optimization and scenario creation. The robustness of the weighting systems was investigated to estimate the variance in simulated results. Validation approaches such as two-way ANOVA, Dunnett's test, and pairwise t-test were executed to determine significance. Additionally, sensitivity analyses were conducted on thematic layers of the model, focusing on those with the greatest variation in results, such as population density, precipitation, and land use/land cover (LULC), to evaluate their impact on the GVI assessment. The uncertainty of the GVI was quantified through the calculation of the consistency ratio (CR) of the AHP matrix (Equation 5.2).

$$CR = (\lambda max - n) / [(n - 1) * RI] \qquad \textbf{(Equation 5.2)}$$

where λmax = maximum eigenvalue of pairwise comparison matrix, n = number of criteria being compared, and Random Index = RI (pre-determined for 7 thematic layers = 1.3) [28]. If the CR is less than or equal to 0.1, the judgments are considered consistent and reliable.

Thematic Layer Scenario Setting and Simulation.

Several scenarios were designed to provide insight into public policy and management (Table 5.4). These scenarios consider source reduction and substitution of thematic layer to predict the potential vulnerable zones for glyphosate. According to the Belize Pesticide Control Board [161], the pesticide application has increased continuously over the years, despite regulation formulated for pesticide reduction. Between 2010 and 2020, the population growth rate of Belize has been fluctuating within a range of 1.3% to 2% [168]. For scenario 1, a population growth rate of 2% was considered to estimate the influence on the spatiotemporal vulnerability mapping in the watershed. Scenarios 2 and 3 were designed such that precipitation would increase and decrease respectively by the average rate of 8%. Historical data from the Belize Meteorological Service show an average year- to-date increase in rainfall for the region of 7.3% [169].

Given the high potential for movement of glyphosate, it is necessary to take the weather into account when applying pesticides and to adhere to best management practices, which include using technologies for drift reduction, applying pesticides at the appropriate time, and avoiding application when the weather is unfavorable. Scenarios 4 and 5 were designed to consider that the target pesticide would increase with respective growth in agricultural production and urbanization. The projected figures are found in accordance with the World Banks's outlook for Belize urbanization and growth [168]. By changing the input of these properties, a new vulnerability map was calculated with these modified models.

Table 5.4. Summary of Scenario settings.

Scenario	Thematic Layer	Description
1	Population Density	Increase by 2%
2	Precipitation	Increase by 8%
3	Precipitation	Decrease by 8%
4	LULC	Increase agricultural lands by 7.2%
5	LULC	Increase urbanization by 2.5%

Results and Discussion

Thematic Layering for Vulnerability Assessment and Spatial Zoning

The findings indicate that the northern and western regions of Belize's watersheds exhibit high infiltration, ranging from 6.1 to 9 (Figure 5.3a). This notable phenomenon can be attributed to the prevalence of leptosol soil profiles in the karst plains, which have a significant impact on pesticide drift and groundwater contamination. Most of the watersheds are categorized as having high infiltration, mainly due to the low saturation co-efficient observed in the soil profiles. This characteristic suggests a potential for leaching and runoff, as water and pesticides can easily move through the soil and aquifer connections in these areas. SDG 6 emphasizes clean water and sanitation, as high infiltration can lead to groundwater contamination and pesticide runoff, compromising water quality for human consumption and ecosystems [38].

In areas with a low slope (0 – 8 degrees), surface runoff is slow, allowing rainwater to percolate more effectively. As a result, these regions are considered low-risk potential

zones for glyphosate, as depicted in Figure 5.3b. On the contrary, areas with high slopes experience higher runoff rates, leading to reduced residence time for rainwater, increased runoff, and limited sorption to the soil. Off-target deposition and runoff-carried pesticide flow can harm nearby vegetation and water bodies. Proper pesticide use can enhance agricultural productivity, but improper drift can cause unintended effects, affecting non-target crops and pollinators, threatening food production and security [139].

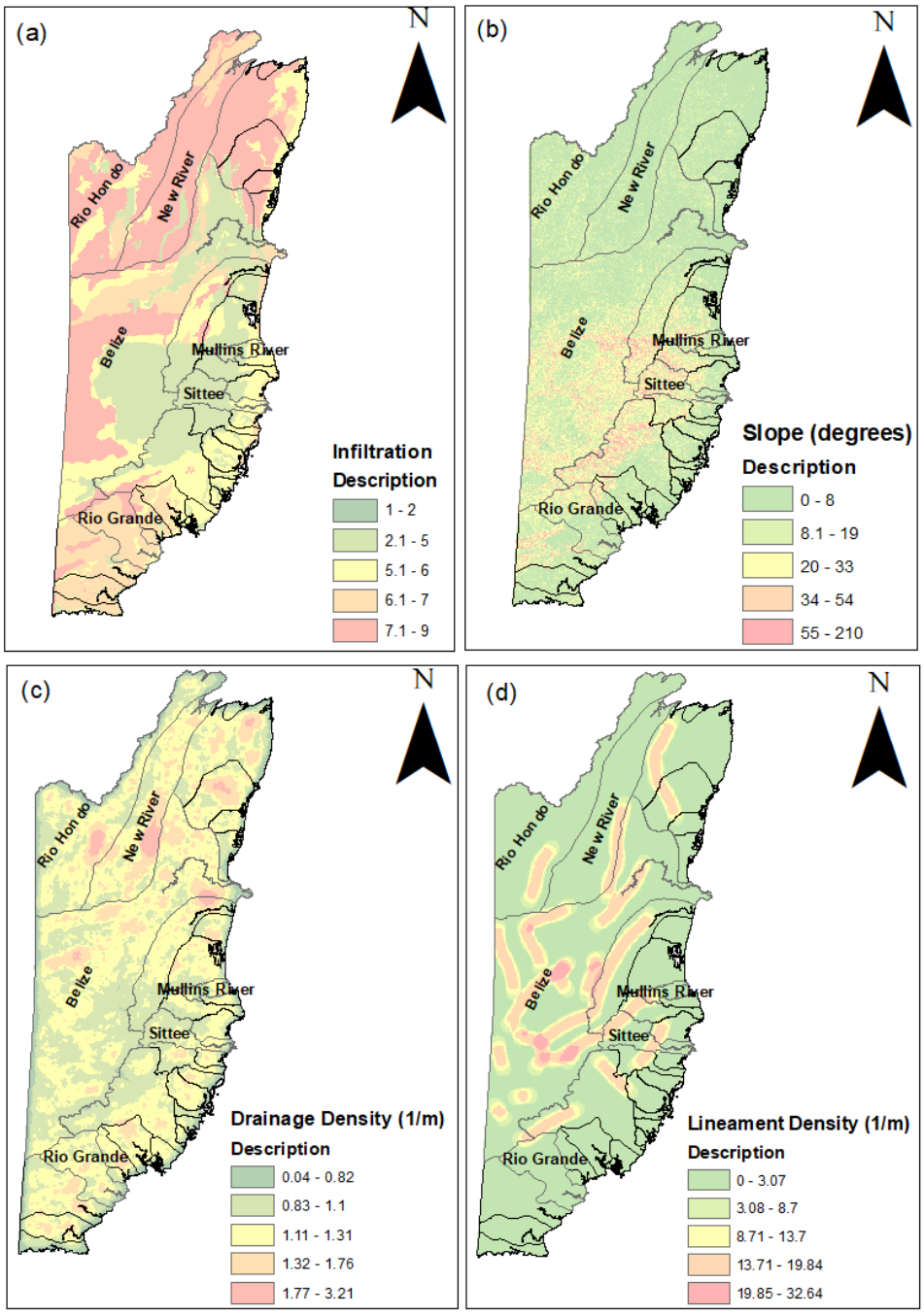


Figure 5.3. (a) Infiltration, (b) slope, (c) drainage density, and (d) lineament density of the watersheds. (Classification: dark green = very low, light green = low, yellow = prone, orange = high, and red = very high).

In the northern regions of the watershed, relatively higher levels of rainfall contribute to increased runoff, allowing for high drainage density (Figure 5.3c). Effective management of drainage density variations plays a critical role in ensuring water quality and sanitation [170]. High drainage density can lead to increased runoff, posing risks to the health of ecosystems.

Understanding and addressing these factors are essential in promoting sustainable water management practices [143]. By controlling pesticide drift and runoff, urban areas can be protected, safeguarding the well-being of residents, and supporting sustainable land use practices. Few areas with the study location were characterized by high lineament density (22 - 33 m^{-1}), which may pose an extreme risk of glyphosate groundwater pollution, while those with low lineament density (0 - 3.1 m^{-1}) present a low risk ($p<0.05$). All lineaments within the watershed illustrated in Figure 5.3d are geomorphic and play a direct role in influencing glyphosate contamination through their impact on water pathways.

The results from Figure 5.4a demonstrate that the north sector of the research region received less rainfall than the remainder ($p<0.05$). The GVI are significant via precipitation dilution, runoff, erosion or leaching [140]. Based on the infiltration map (Figure 5.3a), it is more likely for heavy rainfall to wash off applied glyphosate on plants or sorbed to soil into nearby streams, rivers, or other water bodies causing potential harm and making the water unsafe for human use. The groundwater risk potential was significantly impacted by precipitation due to greater chances for recharging and high runoff ($p<0.05$). Climate change (SDG 13) influences precipitation patterns, potentially affecting glyphosate runoff and leaching dynamics. Mitigating and adapting to climate change can manage its effects on

groundwater contamination, posing risks to urban areas relying on groundwater for drinking and other purposes [171, 172].

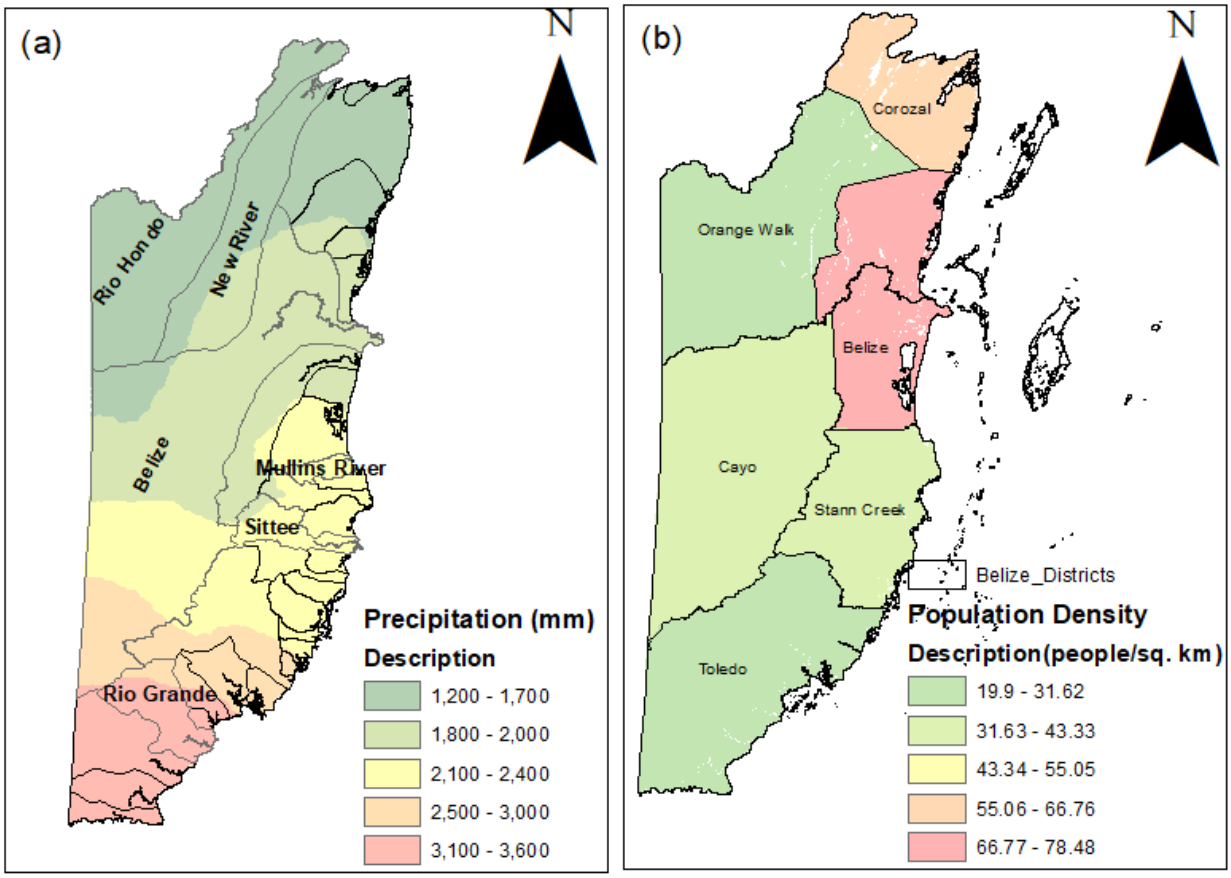


Figure 5.4. (a) Precipitation (b) LULC and (c) population density mapping of the watersheds. (Classification: dark green = very low, light green = low, yellow = prone, orange = high, and red = very high).

The thematic layer classification in Figure 5.4b indicates that Belize district has the highest population density, followed by Corozal and Cayo. Toledo has the lowest population density. These variations in population density are influenced by a range of layers, including historical settlement patterns, economic opportunities, and geographic features [32]. In

areas with high population density, there is often greater use of pesticides to manage pests and insects, such as in urban and suburban areas can result in a higher risk of susceptibility to pesticides for individuals who live or work in these areas [33, 34]. High population density can also lead to increased vulnerability to pesticides through drift or runoff leading to potential susceptibility. The presence of glyphosate in groundwater due to nearby agricultural activities can raise concerns about water safety and sustainable water management for urban communities [35]. The potential health risks in high population density regions with high glyphosate occurrences underline the significance of safe and clean water access for human wellbeing.

The consideration of LULC layer for glyphosate susceptibility and risk investigation is important because the water holding capacity of an area depends on the underlain soil types and their permeability [37, 82] (Figure 5.5). The study area encompasses five distinct land use patterns. Agriculture occupies 24% of the area, while forest covers a substantial 61%, making them the dominant land use types with elevated levels of pesticide residues due to crop cultivation and management practices. Despite urban areas constituting a small portion of the watershed, there exists potential for high pesticide susceptibility, due to the lack of comprehensive monitoring of glyphosate use in urban landscape management. Water bodies account for 3% of the study area, reflecting their limited spatial coverage. According to data from the World Bank [27], Belize's estimated population as of 2021 stands at approximately 408,000 people.

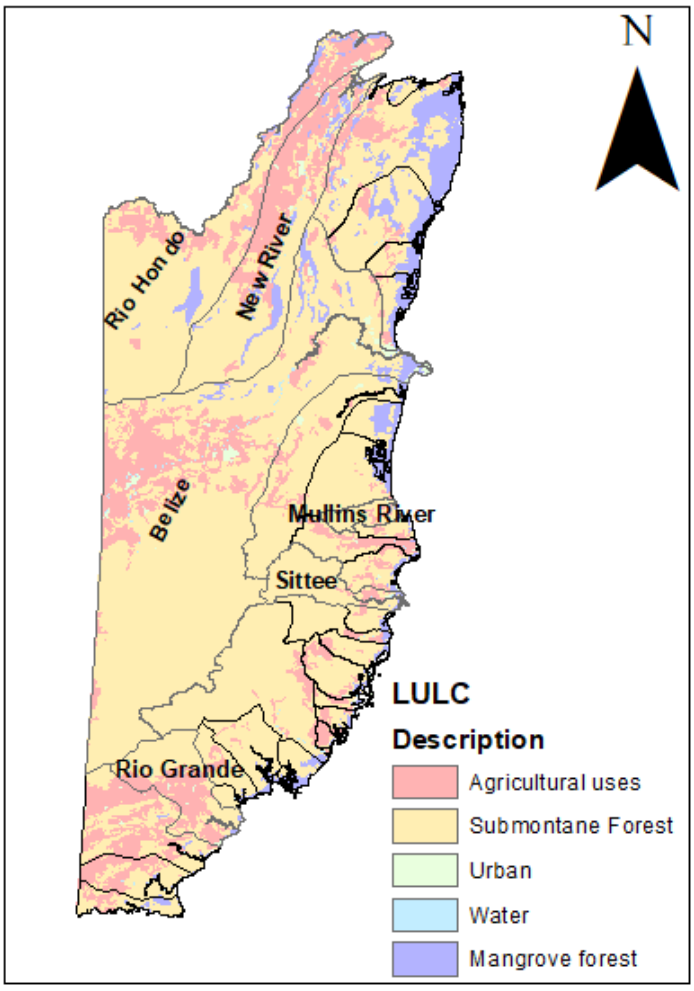


Figure 5.5. LULC mapping of the watersheds (LULC = land use/ land cover).

Glyphosate Vulnerability Mapping

Using the AHP, the *CR* value was < 0.1 showing that the judgments are highly acceptable [25]. The results from the GVI classification reveal varying risk levels across the study area (Figure 5.6). The GVI outcomes reveal that 7% of the area falls under the very low-risk classification, 24% as low-risk, 31% as prone to risk, and 23% and 15% as high-risk and very high-risk areas, respectively. The hotspots of risk are predominantly concentrated in the mid-eastern and southeastern portions of the watershed, including areas like Rio

Grande, Manatee, and Sittee. Among these, the Belize River basin, being the largest in the watershed and rich in biodiversity, was identified as highly prone to pesticide vulnerability. The overlap of biodiversity hotspots, availability of water storage resources, and areas of high-risk pesticide further highlights the urgency of addressing pesticide drift and its potential impact on achieving the SDGs.

The GVI mapping showed that the proportion of moderate and high ecological risk areas increased and posed the biggest threat to individuals in the high populated regions of the watershed indicating a high drift from application regions. This correlation highlights the importance of high scoring and weights associated with the influence of population density. Although high glyphosate usage is usually attributed to high agricultural areas with large-scale intensive farming, the high-risk zones of GVI map were concentrated in economically developed areas. This indicates a shift in sensitivity of the model to effects and influence on the environmental and public health.

A thorough analysis considered potential application sites for glyphosate and the exposed compartment establishing the drift in the lineament contouring. Most places were determined to have karst topography as the highest danger, while 36% of river basins alluvial geological settings were determined to be a higher risk [144]. The primary reason for the high infiltration associated with karst and alluvial geology is the direct contact with routes for the runoff in surface water interaction with groundwater [145, 146]. Understanding the factors influencing runoff in karst and alluvial geology can help develop sustainable agricultural practices that protect water sources and contribute to SDG 6's goal of clean water and sanitation [38, 143]. By recognizing potential risks and adopting

responsible pesticide application methods, farmers and policymakers can develop more sustainable agricultural practices.

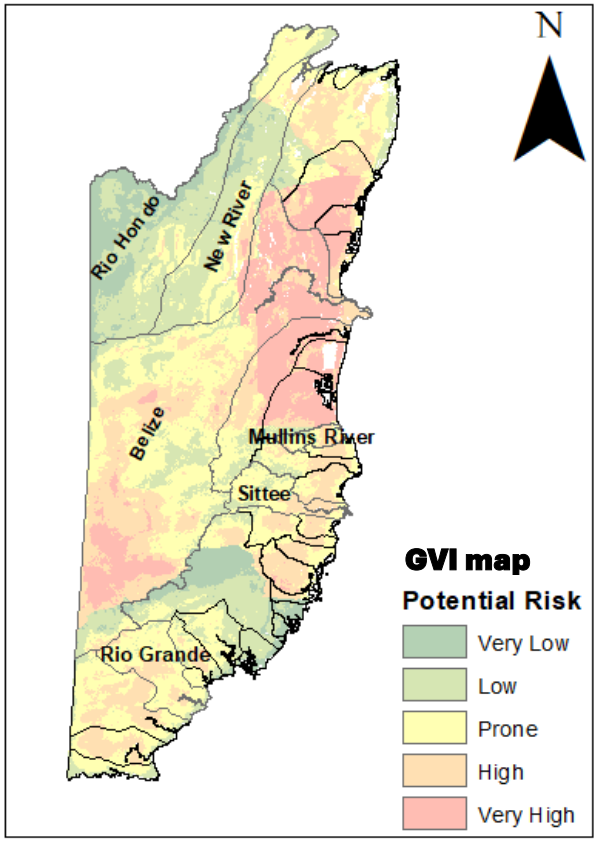


Figure 5.6. GVI spatiotemporal mapping of glyphosate.

Sensitivity Analysis

The model displayed equally notable performance across regions previously categorized as very low, low, and prone to glyphosate susceptibility ($p < 0.05$). Yet, in high and very high-risk areas, results suggested that the AHP model showed excellent robustness in addressing susceptibility. The findings of the AHP models strongly aligned with trends seen in land use/land cover, population density, and precipitation, which are key factors

initiating glyphosate transport within watersheds. The results of the influence of the various layers on the robustness of the selected model, AHP model, revealed that population density, precipitation, and LULC are influential parameters (Table S5.1). No significant changes in classification of risk areas were seen when either the drainage density, slope, infiltration, or lineament density maps were removed individually ($p > 0.05$). The results show that these thematic layers, although affect the movement of glyphosate within the watershed, do not initiate or have significant influence on the susceptibility potential. However, the significant influence of population density, precipitation and LULC ($p< 0.05$) underlines the critical need for measures to resolve challenges highlighted in SDG 3, 6 and 12 (good health and wellbeing through responsible consumption and enhanced water quality and sanitation).

Thematic layer scenario setting and simulation.

A significant increase in GVI was observed for all classification in the watershed for scenario 1 ($p< 0.05$). Thus, a significant increase in total area classified as prone, high, and very high-risk areas was observed ($p< 0.05$). An increase in population density results in closer proximity of households to agricultural fields; thereby, increasing the likelihood of susceptibility to these pesticides via contaminated water sources inhibiting strives to achieve SDG 3 [6]. Monitoring groundwater quality near high population density areas can help to identify potential contamination and inform management strategies. A similar observation was seen for scenario 2. There was a significant increase in GVI throughout the watershed ($p< 0.05$). Increased precipitation via heavy or light rains potential increases the risk of pesticide leaching and runoff by increasing the amount of water that flows through

the soil [38]. No significant shift in classification of the watersheds were seen for scenarios 3, 4, and 5.

Results from pair wise t-test indicated a significant increase in high-risk classification areas within the watersheds for most scenarios compared to original GVI model (Table S5.2). Scenario 1 compared to scenarios 2, 3, 4, and 5 resulted in significant increase of high-risk areas in the watershed ($p< 0.05$). An increase in population density with either changes in precipitation or LULC translates in an increased GVI classification as vulnerability will have a greater effect on biodiversity. This observation is likely attributed to the potential increase in initiating layers of susceptibility via routes that are more likely to cause harm to highly populated regions [166]. Scenario 2 compared to either scenario 4 and 5 resulted in significant increase in high and very high-risk classification and decrease in prone ($p< 0.05$). Increased precipitation leads to runoff from glyphosate application area; this phenomenon implies a high likelihood of drift within the watershed [39]. An increase in urbanization increases the imperviousness of the watershed making runoffs more likely even without increased precipitation leading to high movements of glyphosate from increase pesticide usage due to increased vegetation cover [140]. Results from these scenarios underscore potentially high susceptibility risks for glyphosate within watersheds with population increase, climate change and urban development.

The three and four scenario factorial analysis revealed a significant expansion in areas categorized as prone, high, or very high risk in the GVI mapping of the watersheds ($p< 0.05$). ANOVA results further confirmed a shift in GVI classification across the majority of watersheds due to increased population, intensified agriculture, and either scenario of

precipitation. Particularly, significant portions of the central and southern regions were classified as very high-risk areas. Merely 20% of the watershed demonstrated low or very low risk GVI classifications. This pattern persisted across scenarios involving increased population, urbanization, and precipitation, resulting in 77% of the watershed being classified as highly or very highly vulnerable to glyphosate contamination (Table S5.3).

Best Management Practices for the Use and Handling of Glyphosate

Addressing the environmental and health dangers connected with glyphosate would help to achieve several SDGs, leading to a more sustainable and resilient ecosystem. The study's emphasis on risk assessment and spatial zoning, infiltration, slope, lineament density, drainage density, precipitation, land use, population density, and potential glyphosate risk and vulnerability maps revealed the interdependence of natural and anthropogenic influences. The research investigation highlights the critical need for implementing optimal management practices to effectively accomplish the SDGs. Integrated Pesticide Management strategies that promote holistic and sustainable pesticide management should be encouraged to reduce the usage of glyphosate [6]. Sustainable agriculture, forestry, and water resource management are critical to improving biodiversity, reducing climate change, and protecting water bodies, all of which contribute to SDGs 2, 6, 13, and 15 [7].

Continuous monitoring, research, and cooperation are required to make educated judgments, build successful policies, and put in place long-term practices. A collaborative drive towards reaching the SDGs and establishing a healthier, more sustainable ecology may

be done through prioritizing responsible consumption and production, improving laws, and developing collaborations [147, 148]. It is critical to emphasize the relevance of public awareness and education in engaging the public about the possible hazards of glyphosate and the need for proper handling and disposal methods. Pesticide usage in agriculture and urban areas may be reduced by implementing climate-resilient practices and sustainable urban design, therefore promoting SDGs 11 and 13 [173].

Conclusion

The excessive and improper use of pesticides has detrimental effects on both the environment and human health, presenting obstacles to achieving various Sustainable Development Goals (SDGs). SDG 3, focused on ensuring healthy lives and well-being, and SDG 12, centered on sustainable consumption and production patterns, are particularly susceptible to the negative consequences of pesticide misuse. However, comprehending the intricate patterns of pesticide transportation and susceptibility risks is challenging due to their reliance on hydrological and geological factors. In response to this urgent issue, this study investigated the relationship between glyphosate transportation, the influence of geological structure of Belize's watersheds on glyphosate persistence, and the impact on efforts to achieve the SDGs.

The study developed an Analytic Hierarchy Process (AHP) model to evaluate the spatiotemporal vulnerability of 39 watersheds in Belize to glyphosate. Seven thematic layers were examined and classified into five ranges based on their influence on glyphosate movement. The findings emphasize the need for prompt and coordinated action to mitigate

glyphosate vulnerability and its adverse effects. By adopting best management practices and integrating sustainable solutions into policies and practices, we can protect the environment, promote human health and well-being, and advance progress towards achieving the SDGs, thereby fostering a more resilient and sustainable world. The model identifies 45% of the total watershed area as high risk and aligns with trends observed in land use/land cover, population density, and precipitation, which consistently demonstrate that glyphosate is transported to the mid and southern regions.

The study presents models that help environmental agencies manage pesticide usage and implement interventions to protect agriculture, animal, and human health. These models help stakeholders select optimal management approaches, safeguard water quality, and preserve ecological balance within watersheds. They also provide valuable resources for environmental agencies to understand the spatial dynamics of pesticide application and the influential layers affecting pesticide movement, ensuring sustainable and responsible practices for the sustained achievement of SDGs.

CONCLUSION

For research question 1) How do novel detection techniques and analytical tools contribute to enhancing our ability to monitor glyphosate residues in various water sources, and what are the implications of these findings for environmental and public health management? This research developed two analytical methods for the detection of glyphosate, glufosinate and aminomethylphosphonic acid (AMPA) in water matrices. Key findings from both studies include but are not limited to the influence of liquid chromatography separation parameters such as pH, acidification, mobile phase gradient and type of chromatographic column. The first method development focused on optimizing critical variables for optimal derivatization using a 24-factorial design, demonstrating that complete derivatization significantly depends on the inclusion of borate buffer to create the alkaline conditions necessary for aminolysis. Additionally, the study found that the inclusion of ethylenediaminetetraacetic acid (EDTA) was critical to minimize metallic chelation and ensure reproducible retention times and peaks. The concentration of the derivatization agent and the derivatization time also played crucial roles in achieving complete derivatization. The optimized method demonstrated sensitivity and precision in quantifying these compounds, addressing the challenges associated with their detection due to their lack of chromophores and zwitterionic nature. The effect of solid phase extraction as a preconcentration step after derivatization of glyphosate, glufosinate and AMPA was highlighted in the second method developed. The optimized experimental conditions for extraction include the use of offline Oasis SPE polymeric-based phase sorbents, such as Oasis MCX and Oasis WAX cartridges. The extraction process involves conditioning the cartridges with 2 mL of MeOH, equilibrating with DI water, loading 2 mL of the derivatized sample onto

the cartridge at a rate of 1 mL/min, washing with 5% NH_4OH, and eluting the analytes at a rate of 1 mL/min using organic solvents. These conditions were found to enhance the recovery and detection of glyphosate, glufosinate, and AMPA in water samples, hence the detection limits of this method for the target analytes were below the maximum contaminant levels prescribed by the World Health Organization's (WHO) International Agency for Research on Cancer (IARC).

For research question 2) What are the spatiotemporal dynamics governing the transport and distribution of glyphosate, glufosinate, and AMPA within watersheds, and how can remote sensing technologies and groundwater interconnectivity models inform strategies for mitigating environmental impacts? This study used two watersheds, Kentucky River basin in Kentucky USA and the Belize watershed comprising of the 39 basins in Belize, to investigate the factors that influence the vulnerability of water resources and reservoirs to potential glyphosate contamination. The Groundwater Vulnerability Index (GVI) of the watershed were heavily influenced by population density, climate change and land use / land cover (LULC). The majority of the watershed experienced higher GVI due to heavy urbanization and agricultural activities which affected the groundwater recharge and ecological balance. The high population density regions correlated with increased pesticide use and higher potential risk of groundwater contamination. Scenarios of increased precipitation due to climate change resulted in high runoffs from application sites and increasing the GVI of unintended locations or areas with low usage of the glyphosate-based pesticides. The presence of karst in both watersheds significantly increased the GVI by promoting rapid infiltration and widespread transport of pesticides through its network of

swallets, conduits and channels. This comprehensive analysis underscores the complex interplay between natural and human factors in pesticide transport and groundwater contamination, advocating for integrated management strategies to ensure sustainable water resource use.

For research question 3) What socio-economic and environmental justice factors influence the interplay between agricultural practices, glyphosate usage, and societal equity considerations, and how can this understanding inform the development of sustainable management practices tailored to diverse rural and urban landscapes? Two critical examinations were conducted to ascertain the socio-economic and environmental justice trends related to glyphosate. First, a correlation of the GVI was undertaken to evaluate the disparities and tendencies of potential contamination of water resources due to glyphosate usage. All federally designated opportunity zones fell into high vulnerability areas, indicating the need for equitable economic opportunities and comprehensive solutions to address the environmental injustices these communities face. Marginalized communities, particularly low-income and BIPOC groups, were found to be disproportionately affected. The second investigation evaluated the effects of glyphosate overuse and runoffs on the attainment of the United Nations (UN) Sustainable Development Goals (SDGs) as prescribed in Rio de Janeiro in 2012. Negative impact correlations were seen on both the environment and human health, hindering the achievement of Sustainable Development Goals (SDGs) 3 (health and well-being) and 12 (sustainable consumption and production).

The findings from these investigations into the fate, transport, and impacts of glyphosate, glufosinate and AMPA suggest that the use, management, and monitoring of

glyphosate-based pesticides needs significant policy directions overhaul to effectively minimized the interruptions and potential negative impacts on the quality of life of the ecosystem and built environment. They provide a significant framework for the intellectual merit arguments needed to address the several critical aspects of glyphosate, glufosinate, and AMPA analysis, usage, and environmental impacts. The interdisciplinary approach adopted in the research approach developed targeted strategies for mitigating the complex socio-economic and environmental justice factors, highlighting the disproportionate effects of glyphosate usage on marginalized communities. The findings demonstrate the intellectual merit of studying glyphosate-based herbicides from multiple perspectives, addressing knowledge gaps, and providing actionable insights. This research contributes to understanding glyphosate's environmental fate and its implications for ecosystem health and societal well-being, promoting sustainable agricultural practices and environmental stewardship.

With public outcry and focus on the impact of glyphosate residues on water sources and their transport dynamics, findings from this work setup the tools needed to improve the management strategies to mitigate contamination risks and protect human health and ecosystem integrity. Empowerment of users, stakeholders, and the community is essential to development and adoption of an integrated use and management plan for glyphosate and glufosinate. This research, as a framework for equitable policy interventions, identifying disparities in groundwater vulnerability and assessing the overuse on marginalized communities, describes the following recommendations urgently needed to safeguard our environment are met. The recommendations are as follows:

- The use of newly developed, novel, sensitive, and precise detection methods in better monitoring of glyphosate, glufosinate and AMPA residues is crucial for public health and environmental safety.
- The development of an integrated management plan backed by the understanding of the spatiotemporal dynamics of glyphosate transport to inform on strategies needed for contamination mitigation and water resources protection.
- Engagement and revision of programs to address the socio-economic and environmental justice issues required for inclusive and equitable sustainable development.

The interdisciplinary nature of the research fosters collaboration between scientists, policymakers, and stakeholders from diverse backgrounds, promoting knowledge exchange and translating findings into actionable policies. This research contributes to promoting environmental sustainability, protecting public health, advancing social equity, and informing evidence-based policymaking. By addressing the challenges posed by glyphosate-based herbicides, it contributes to a more resilient and sustainable future for current and future generations.

Future research on glyphosate-based herbicides should extends beyond their individual effects, encompassing investigations into their interactions with other agricultural chemicals,, exploration of alternative formulations that minimize environmental impacts while maintaining efficacy, comparative assessments of the risks associated with proposed glyphosate substitutes, and examinations of regulatory policies and governance frameworks governing pesticide use. The exploration of alternative formulations would

ultimately lead to evidence-based policy interventions for environmental protection and safeguard public health.

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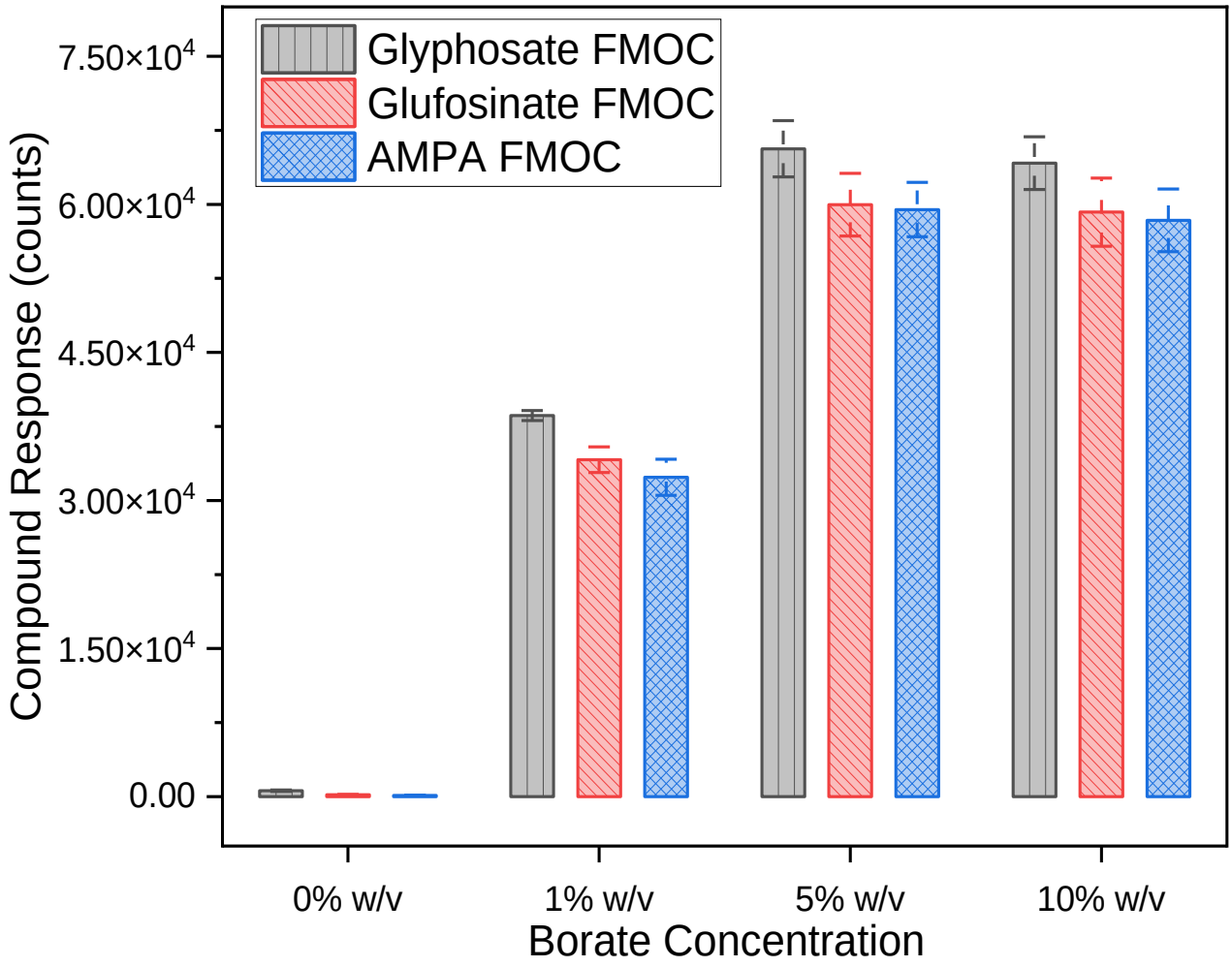
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APPENDIX A

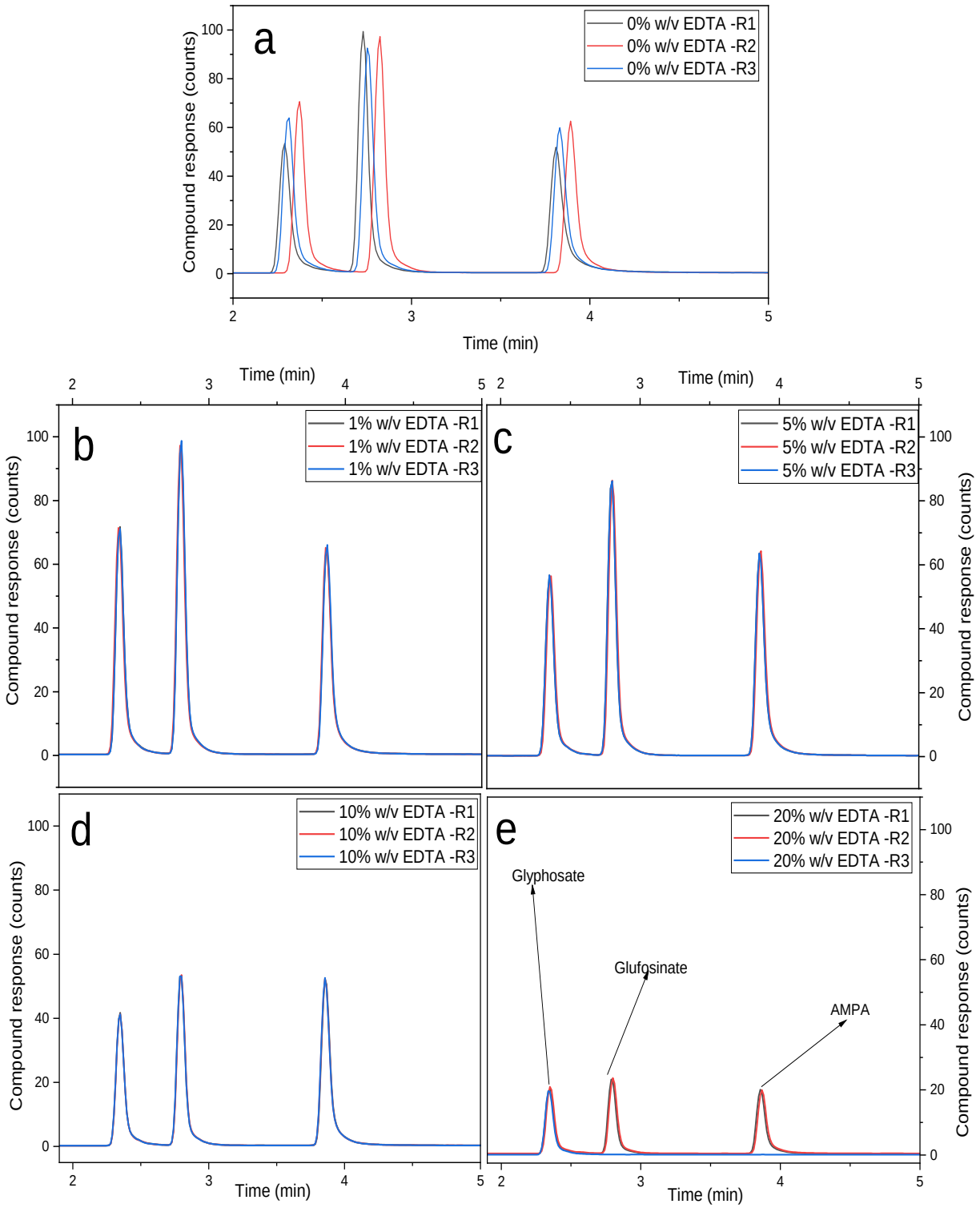
ADVANCED LIQUID CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY METHOD FOR QUANTIFYING GLYPHOSATE, GLUFOSINATE, AND AMINOMETHYLPHOSPHONIC ACID USING PRE-COLUMN DERIVATIZATION

This chapter was published in the peer-reviewed journal ACS ES&T Water and appears as published. The citation of the article is:

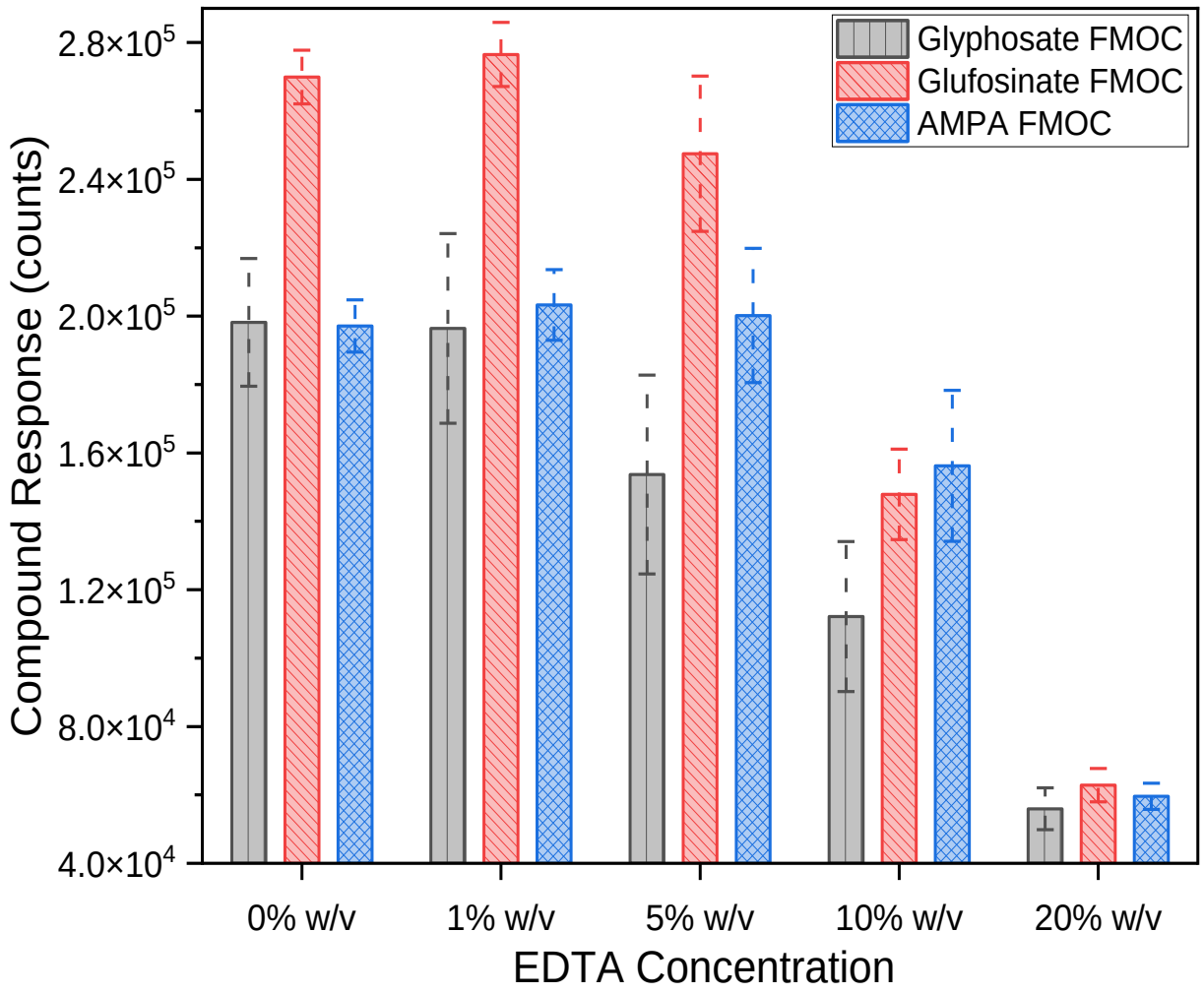
Martin, P. J., He, K., Blaney, L., & Hobbs, S. R. (2023). Advanced Liquid Chromatography with Tandem Mass Spectrometry Method for Quantifying Glyphosate, Glufosinate, and Aminomethylphosphonic Acid Using Pre-Column Derivatization. ACS ES&T Water, 3(8), 2407–2414. https://doi.org/10.1021/acsestwater.3c00094



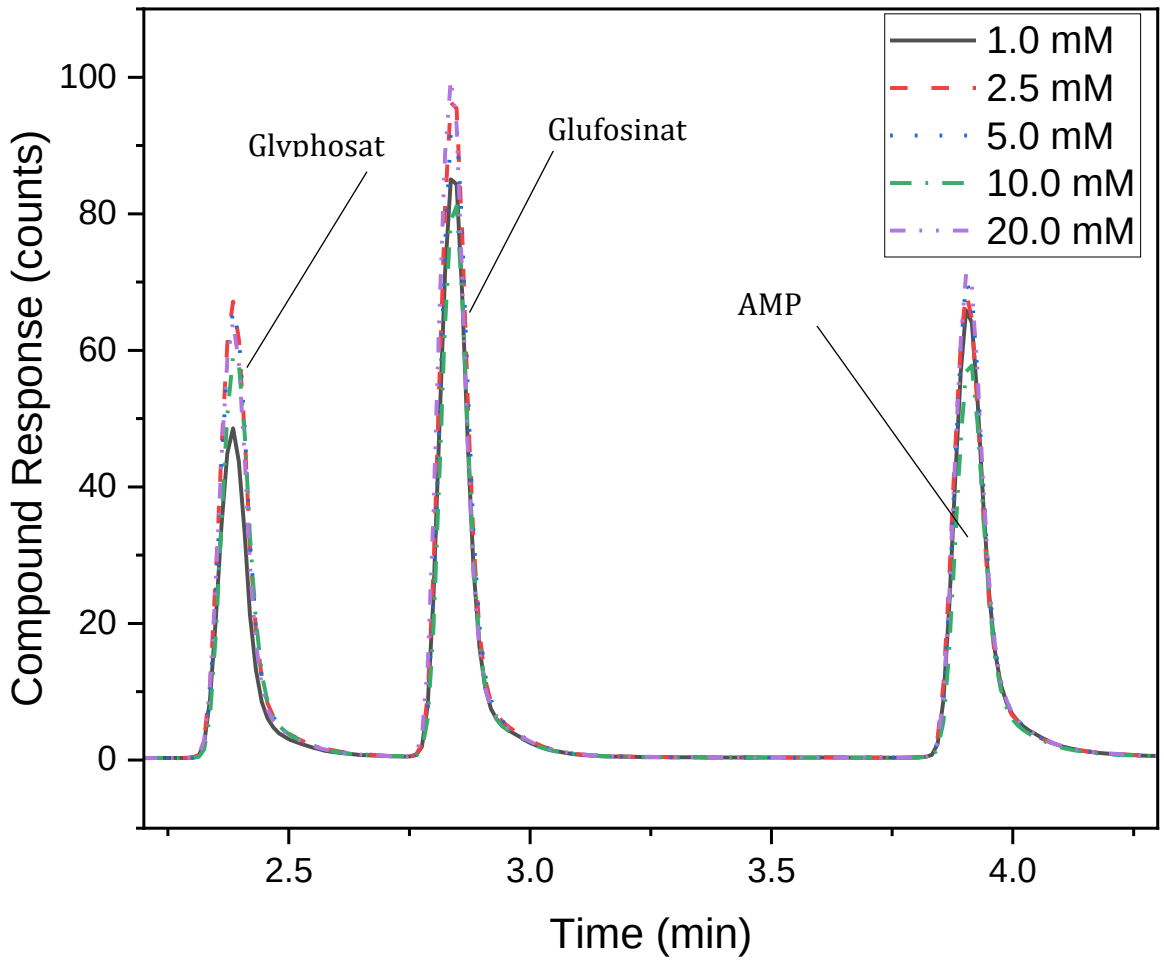
Supporting Information: Figure S1 - Chromatographic response of target compounds with varied borate concentrations (n=3, error bar = standard deviation).



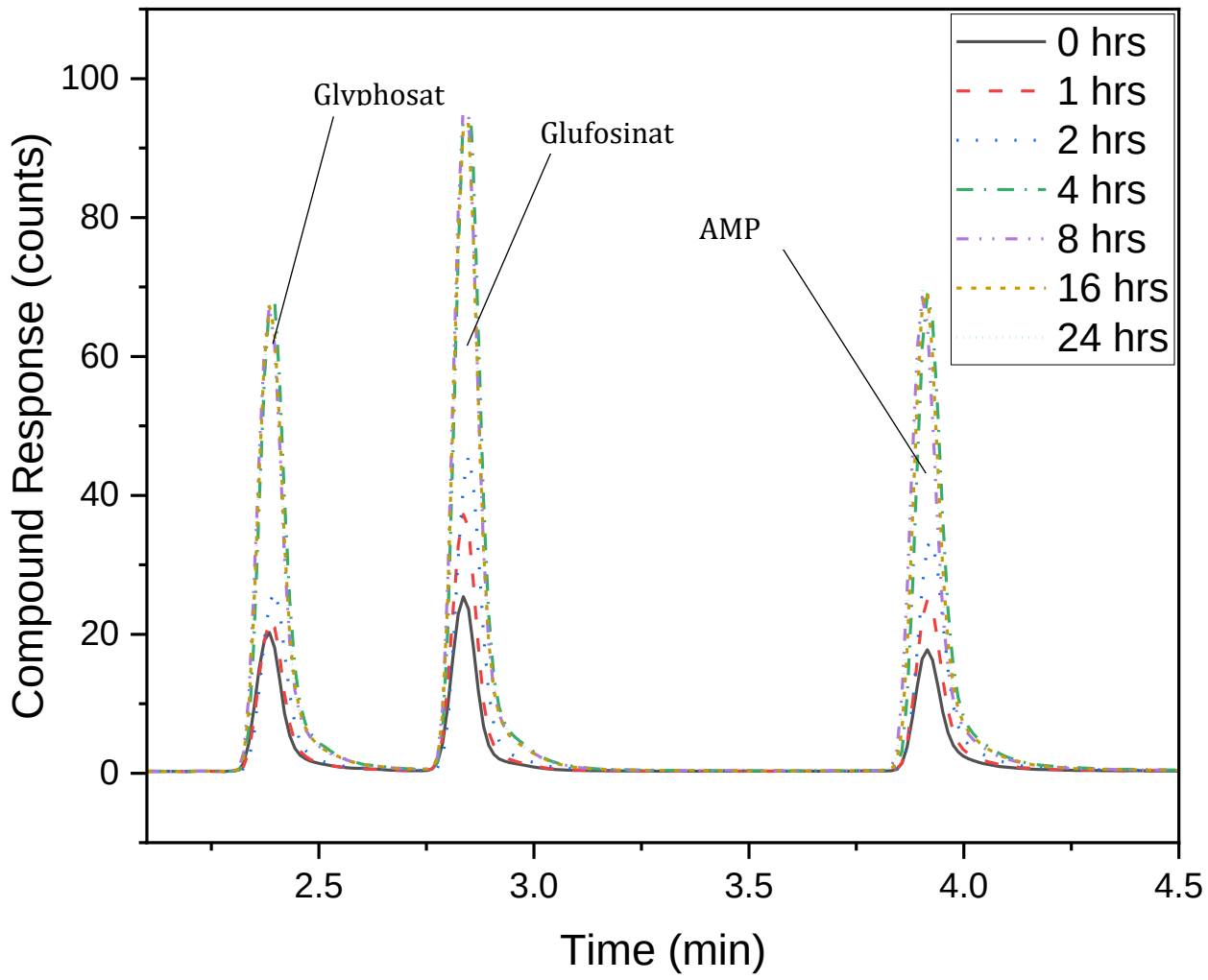
Supporting Information: Figure S2 - Highlights (a)variation in retention times of target compounds with no EDTA addition and (b-e) minimal variation with the addition of EDTA.



Supporting Information: Figure S3 - Chromatographic response of target compounds with varied EDTA concentrations (n=3, error bar = standard deviation).



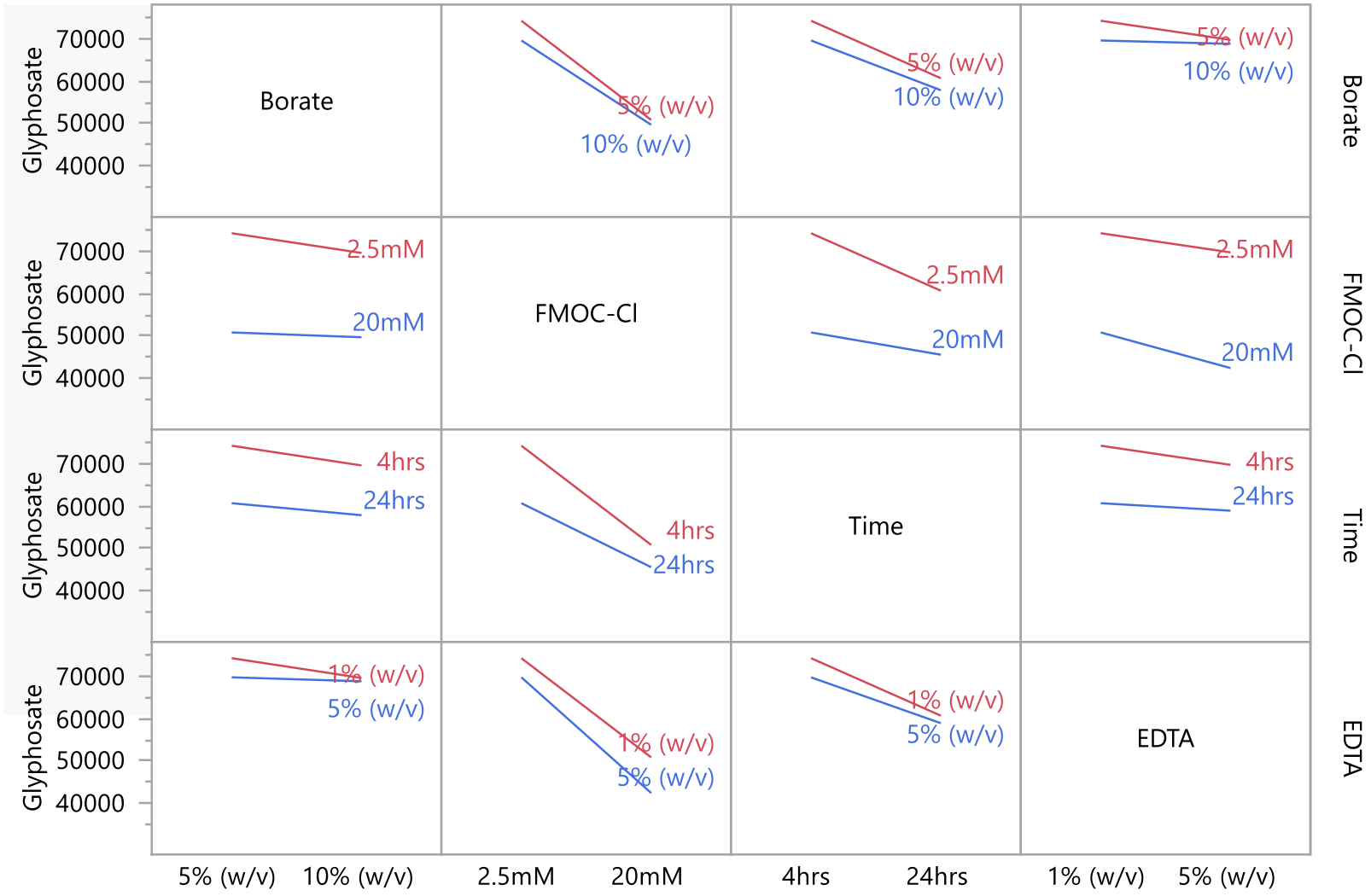
Supporting Information: Figure S4 - Chromatographic response of target compounds with varied FMOC concentrations.



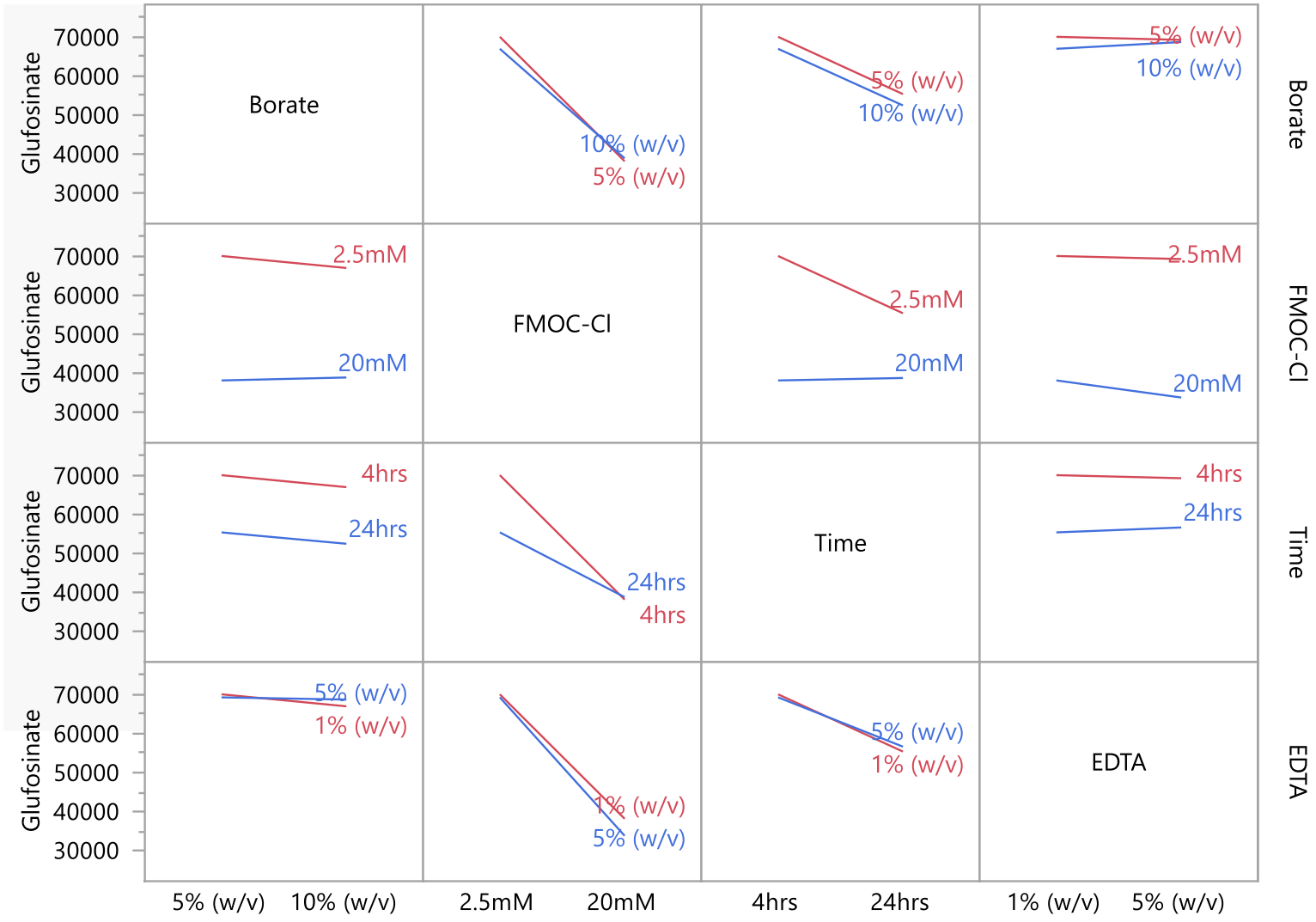
Supporting Information: Figure S5 - Chromatographic average response of target compounds with varied derivatization times.

Supporting Information: Table S1 - Desirability of optimal conditions for the complete derivatization of glyphosate, glufosinate, and AMPA in terms of reaction time and derivatizing agent concentration

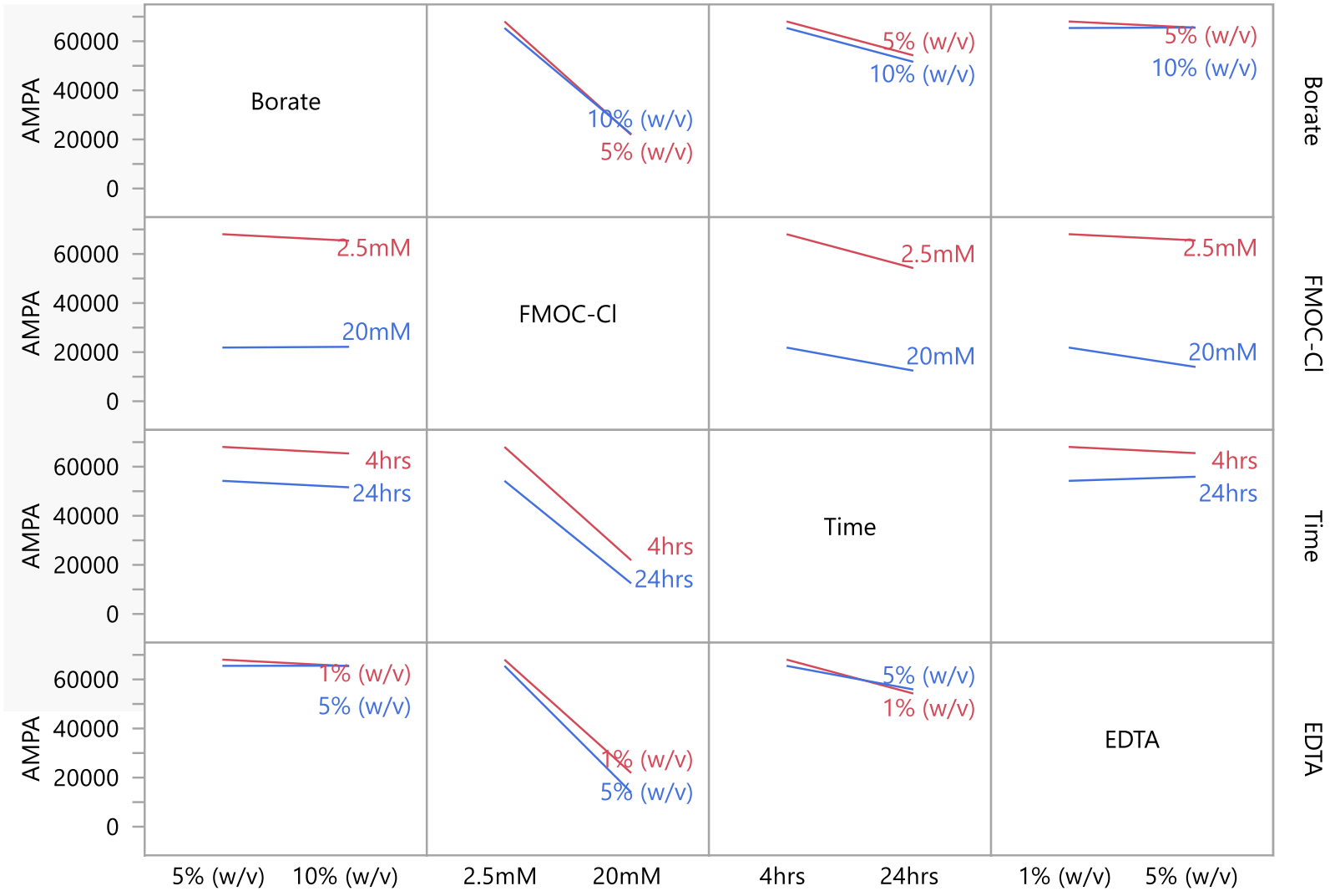
Borate (w/v)	FMOC-Cl (mM)	Derivatization time (hrs)	EDTA (w/v)	Total Desirability	Glyphosate Desirability	Glufosinate Desirability	AMPA Desirability
5%	2.5	4	1%	**0.93**	0.96	0.87	0.95
5%	2.5	4	5%	**0.88**	0.85	0.85	0.92
5%	2.5	24	1%	**0.65**	0.63	0.56	0.76
5%	2.5	24	5%	**0.65**	0.59	0.58	0.78
5%	20	4	1%	**0.31**	0.41	0.22	0.33
5%	20	4	5%	**0.20**	0.23	0.14	0.23
5%	20	24	1%	**0.25**	0.29	0.24	0.22
5%	20	24	5%	**0.18**	0.17	0.19	0.17
10%	2.5	4	1%	**0.86**	0.85	0.81	0.92
10%	2.5	4	5%	**0.86**	0.83	0.84	0.92
10%	2.5	24	1%	**0.59**	0.57	0.50	0.72
10%	2.5	24	5%	**0.65**	0.61	0.58	0.79
10%	20	4	1%	**0.31**	0.38	0.24	0.34
10%	20	4	5%	**0.25**	0.28	0.20	0.27
10%	20	24	1%	**0.26**	0.31	0.25	0.22
10%	20	24	5%	**0.24**	0.27	0.26	0.21



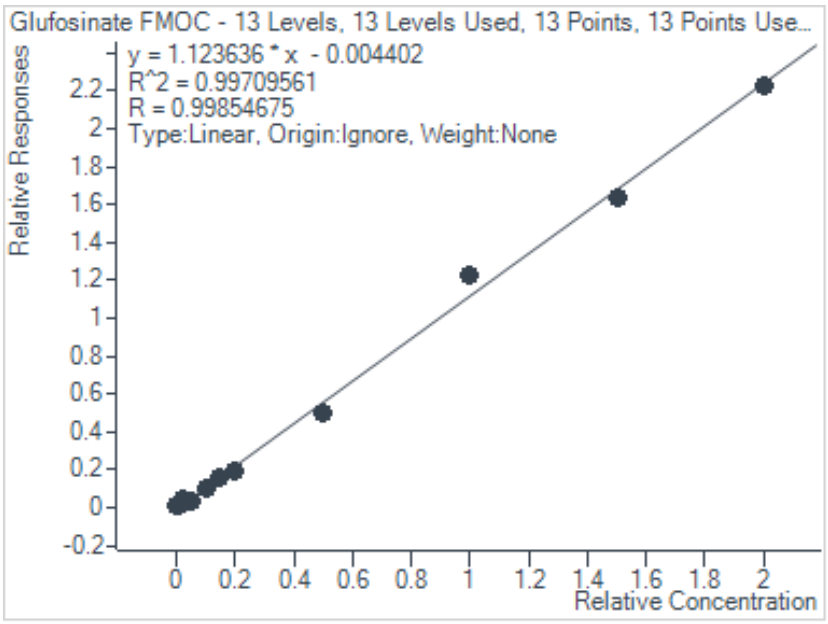
Supporting Information: Figure S6a - Interaction profile of the various derivatization factors for glyphosate.



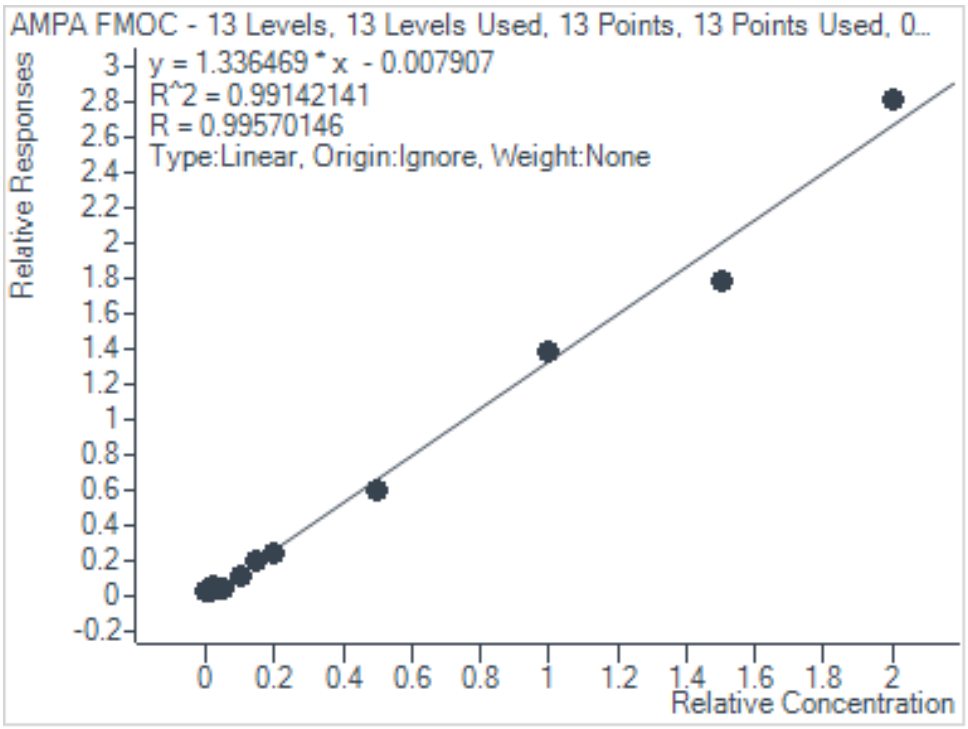
Supporting Information: Figure S6b - Interaction profile of the various derivatization factors for Glufosinate.



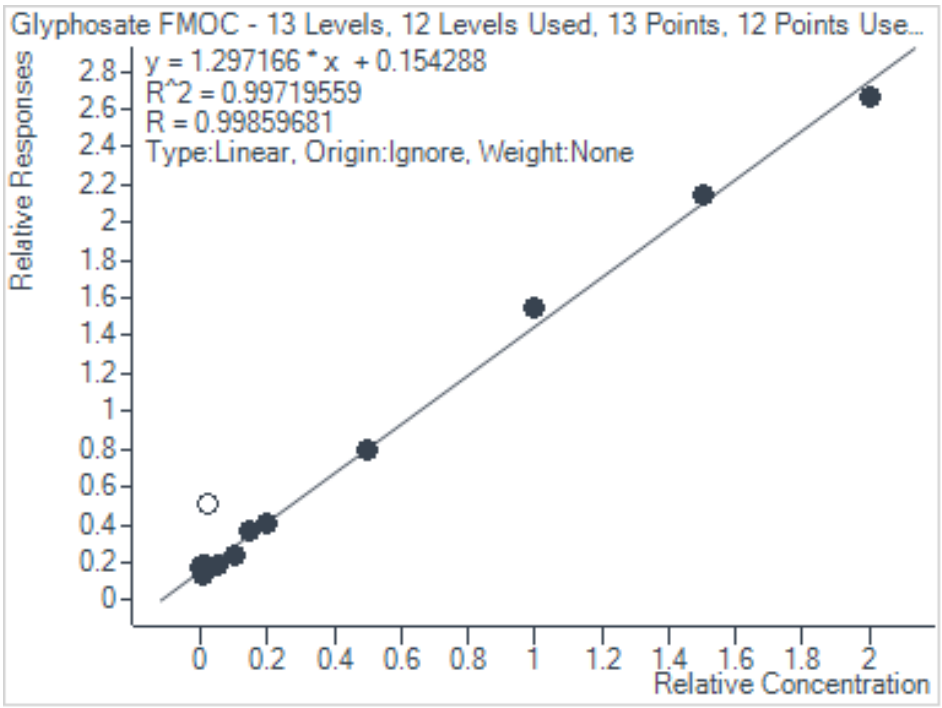
Supporting Information: Figure S6c - Interaction profile of the various derivatization factors for AMPA



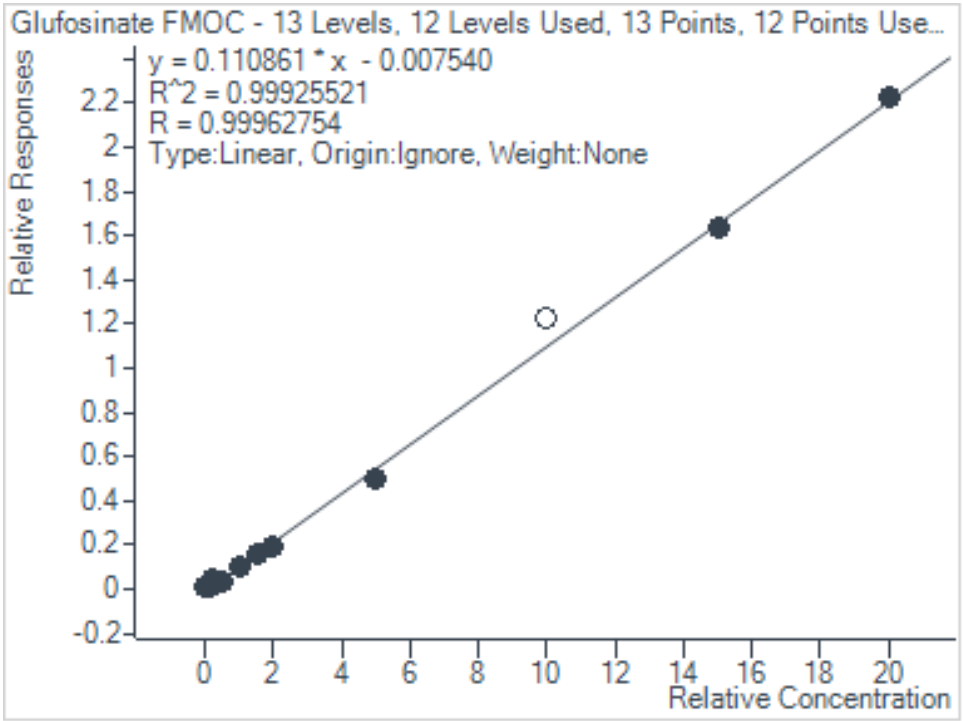
Supporting Information: Figure S7 - Calibration curve for Glufosinate (low level)



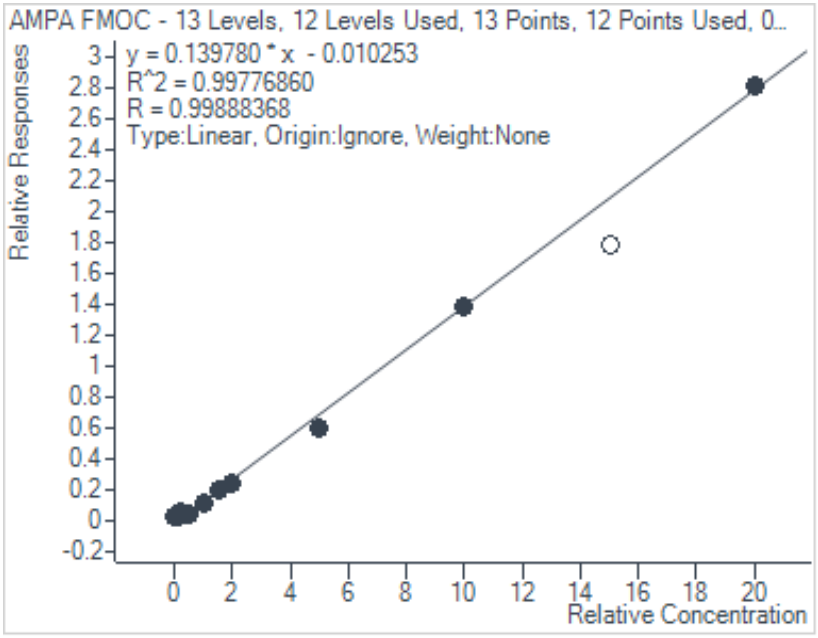
Supporting Information: Figure S8 - Calibration curve for AMPA (low level)



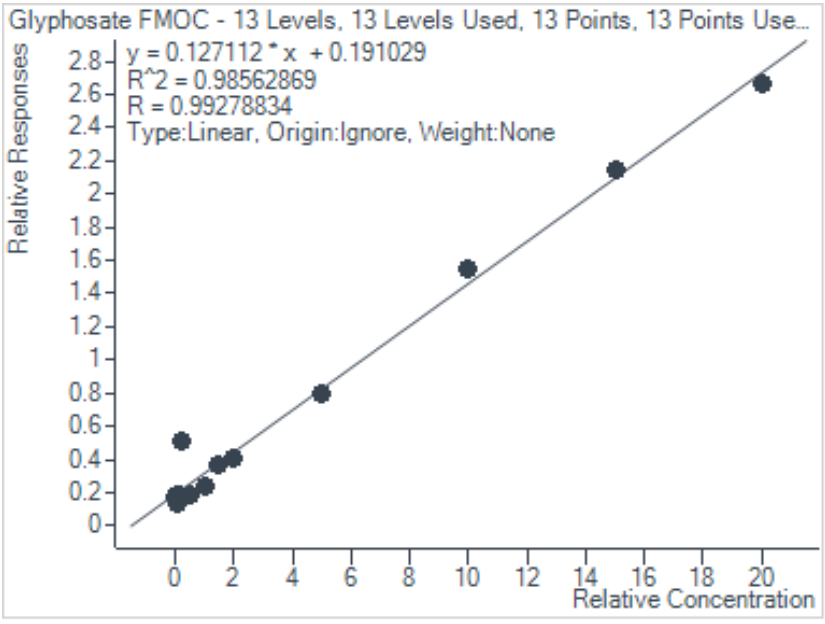
Supporting Information: Figure S9 - Calibration curve for Glyphosate (low level)



Supporting Information: Figure S10 - Calibration curve for Glufosinate (high level)



Supporting Information: Figure S11 - Calibration curve for AMPA (high level)



Supporting Information: Figure S12 - Calibration curve for Glyphosate (high level)

APPENDIX B

Matrix Effect (ME) = $\frac{R_{solvent\ standard}}{R_{post-extracted\ spiked\ samples}} \times 100$ (Equation S3.1)

where $R_{solvent\ standard}$ = response of the solvent standard sample and $R_{post\text{-}extracted\ spiked\ samples}$ = post-extracted spiked sample in ultrapure water.

Table S3.1. Detailed information about target analytes [174]

Target Analyte	Common Trade Name	CAS Number	Molecular Weight (g/mol)	pKa	Log P
Glyphosate	Roundup	1071-83-6	169.1	0.8, 2.3, 6.0 and 11.0	-2.8
Glufosinate	Liberty	51276-47-2	181.1	2.9 and 9.8	0.1
AMPA	-	1066-51-9	111.1	2.35, 5.9 and 10.8	-4.7

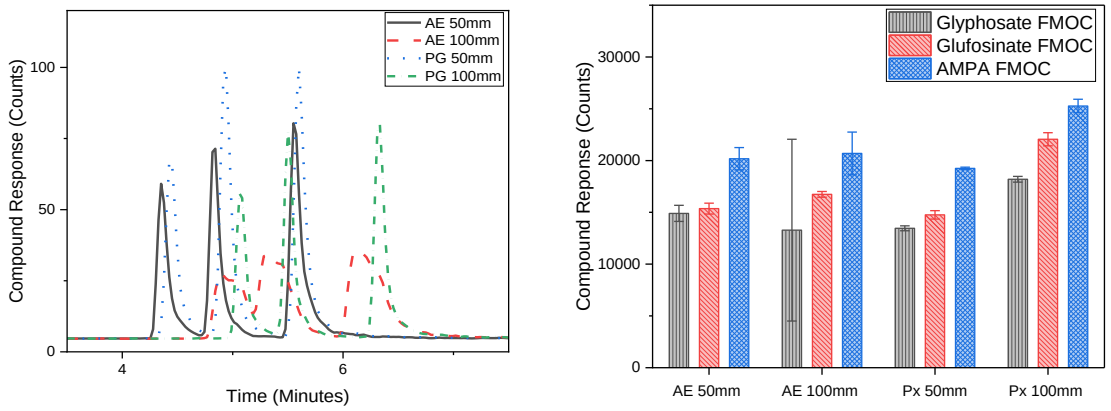


Figure S3.1. Effects of column length and packing material on the chromatographic separation of target analytes (AE = Agilent Eclipse, PG = Phenomenex Gemini).

Table S3.2. ANOVA results for effects of column length and packing material on the chromatographic separation of target analytes (AE = Agilent Eclipse, PG = Phenomenex Gemini).

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**	**Prob > F**
Name	3	64769834	21589945	33.0570	<.0001
Error	8	5224901	653112.59		
C. Total	11	69994734			

Level	**Number**	**Mean**	**Std Error**	**Lower 95%**	**Upper 95%**
Agilent Eclipse 50mm	3	20173.2	466.59	19097	21249
Agilent Eclipse 100mm	3	20685.5	466.59	19610	21761
Phenomenex 50mm	3	19244.4	466.59	18168	20320
Phenomenex 100mm	3	25265.4	466.59	24189	26341

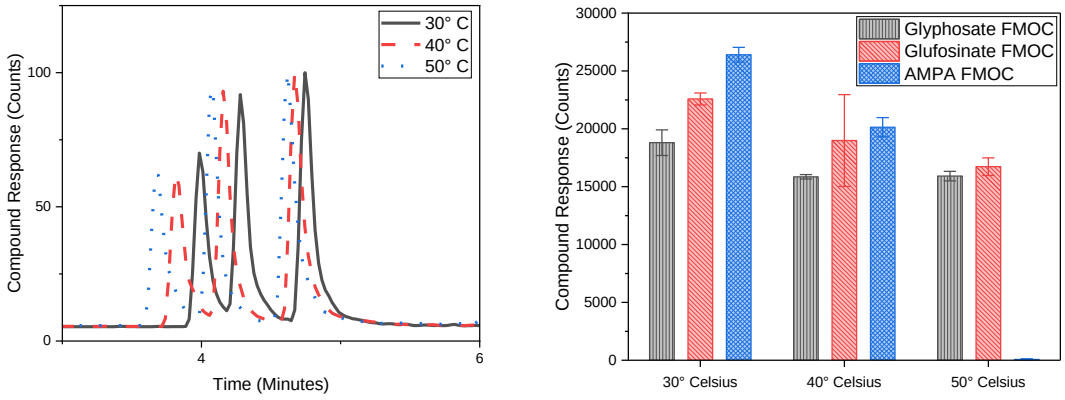


Figure S3.2. Effects of column temperature on the chromatographic separation of target analytes.

Table S3.3. ANOVA results for effects of column temperature on the chromatographic separation of target analytes.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Name	2	17020164	8510082	39.9744	0.0003
Error	6	1277330	212888		
C. Total	8	18297494			

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Temperature 30 degree Celsius	3	18799.5	266.39	18148	19451
Temperature 40 degree Celsius	3	15852.0	266.39	15200	16504
Temperature 50 degree Celsius	3	15913.5	266.39	15262	16565

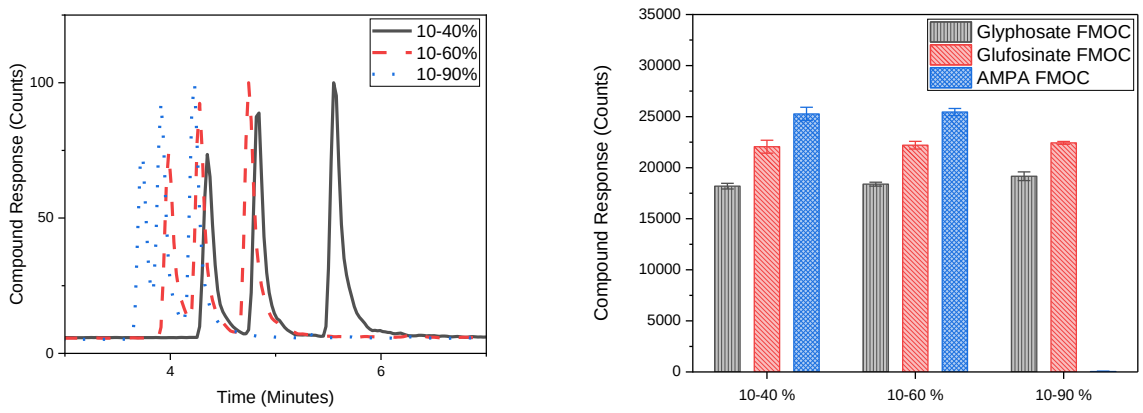


Figure S3.3. Effects of mobile phase B gradient (acetonitrile) on the chromatographic separation of target analytes.

Table S3.4. ANOVA results for effects mobile phase B gradient (acetonitrile) on the chromatographic separation of target analytes.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Name	2	1134972444	567486222	3504.502	<.0001*
Error	6	971583.832	161930.64		
C. Total	8	1135944028			

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Mobile phase B 10-40%	3	26396.9	232.33	25828	26965
Mobile phase B 10-60%	3	20137.4	232.33	19569	20706
Mobile phase B 10-90%	3	70.2	232.33	-498	639

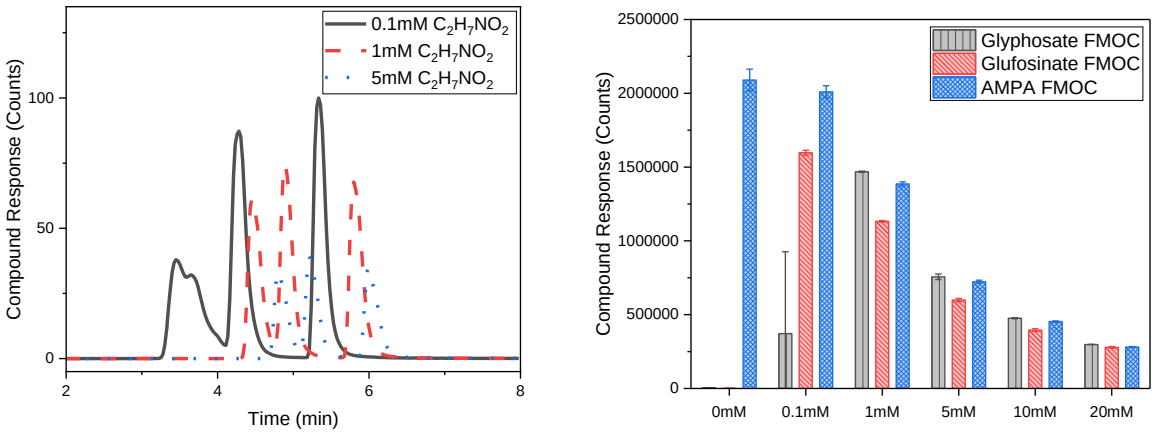


Figure S3.4. Effects of mobile phase A acidification with $C_2H_7NO_2$ on the chromatographic separation of target analytes (High standard deviation: Ghost peak observed for 0.1 mM which correlates with Figure 4)

Table S3.5. ANOVA results for effects of mobile phase A acidification with $C_2H_7NO_2$ on the chromatographic separation of target analytes (High standard deviation: Ghost peak observed for 0.1 mM which correlates with Figure 4)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Name	3	46545068	15515023	1.7969	0.02256
Error	8	69073556	8634194.5		
C. Total	11	115618624			

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
0.1mM	3	14900.1	1696.5	10988	18812
1mM	3	18191.2	1696.5	14279	22103
5mM	3	13684.3	1696.5	9372	17196
10mM	3	13455.8	1696.5	9544	17368

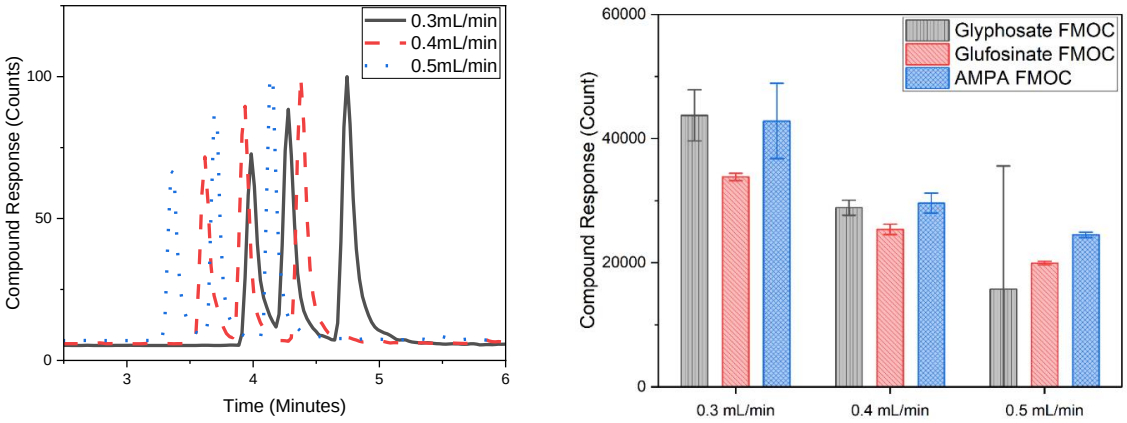


Figure S3.5. Effects of column flowrate on the chromatographic separation of target analytes

Table S3.6. ANOVA results for effects of column flowrate on the chromatographic separation of target analytes

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Name	2	52276365	26138183	10.6286	0.0107
Error	6	14755333	2459222.1		
C. Total	8	67031698			

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Flowrate 0.3ml/min	3	22581.8	905.40	20366	24797
Flowrate 0.4ml/min	3	18986.1	905.40	16771	21202
Flowrate 0.5ml/min	3	16729.1	905.40	14514	18945

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F

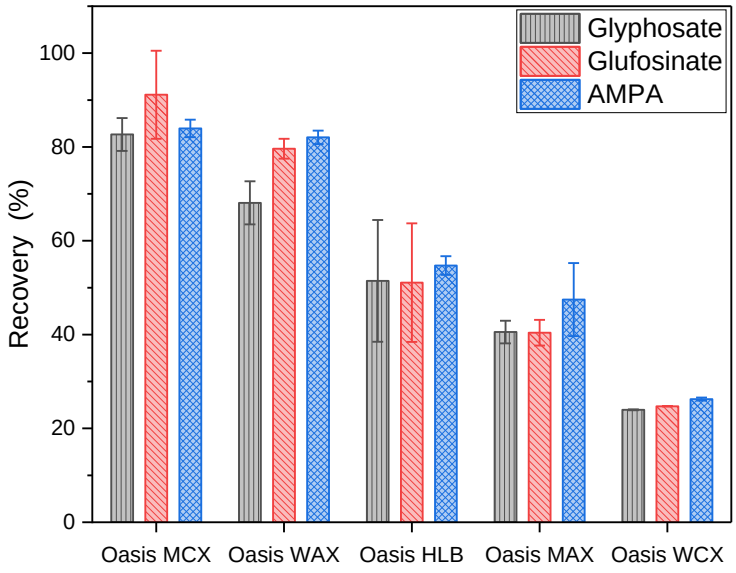


Figure S3.6. Effects of cartridge packing type on the chromatographic separation of target analytes.

Table S3.7. ANOVA results for effects of cartridge packing type on the chromatographic separation of target analytes.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Name	3	99494624	33164875	326.8850	<.0001
Error	8	811659	101457.33		
C. Total	11	100306283			

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Oasis WAX	3	15356.8	183.90	14933	15781
Oasis HLB	3	16736.6	183.90	16313	17161
Oasis MAX	3	14757.3	183.90	14333	15181
Oasis MCX	3	22056.8	183.90	21633	22481
Oasis WCX	3	13455.8	1696.5	9544	17368

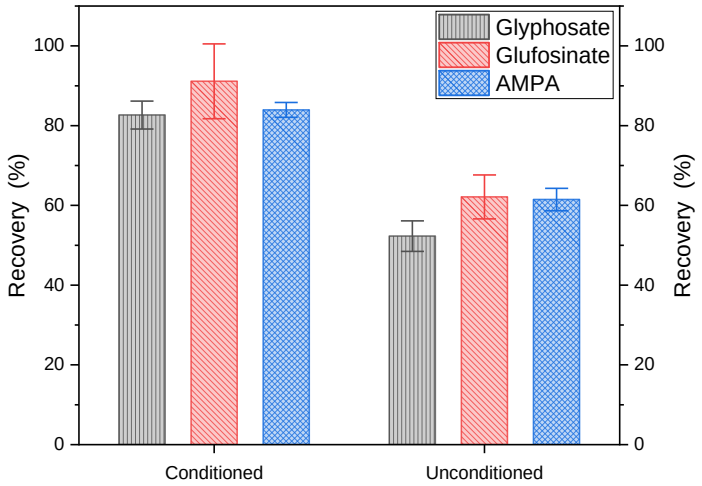


Figure S3.7. Effects of cartridge conditioning on the chromatographic separation of target analytes.

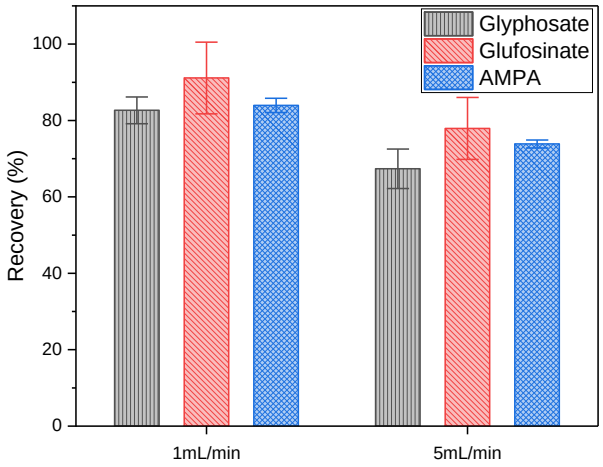


Figure S3.8. Effects of loading flowrate with 2 mL volume on the chromatographic separation of analytes

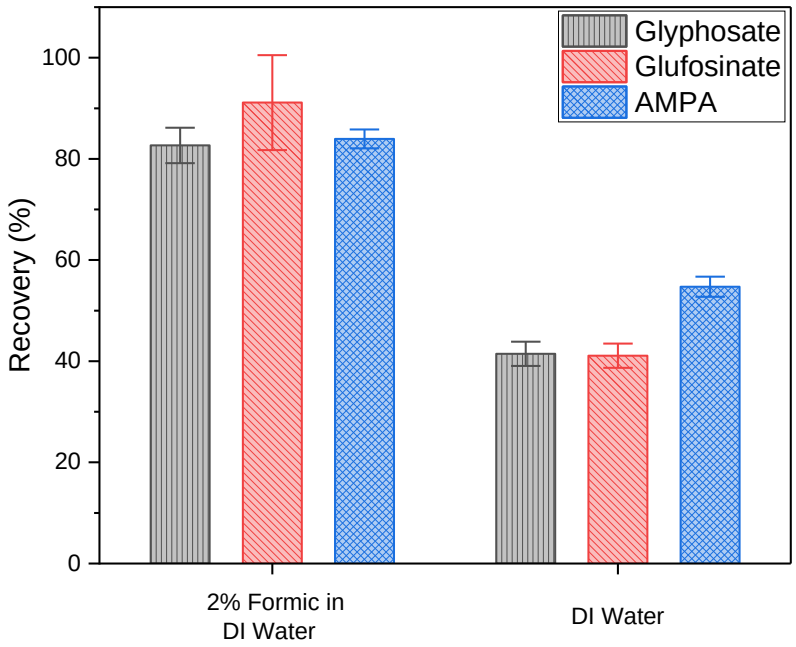


Figure S3.9. Effects of washing solvent composition with 1 mL volume on the chromatographic separation of target analytes.

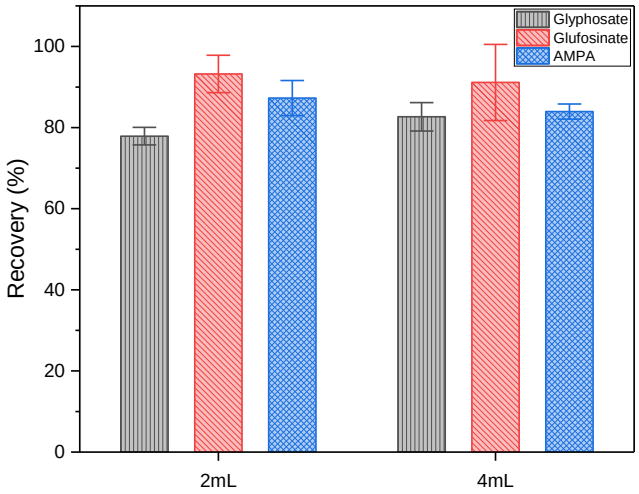


Figure S3.10. Effects of elution volume on the chromatographic separation of target analytes.

Table S3.8. Recoveries for target analytes at spike levels.

Spike levels (µg/L)	Recovery (%)		
	Glyphosate	Glufosinate	AMPA
1.0	88 ± 0.11	93 ± 0.18	90 ± 0.23
2.5	95 ± 0.05	96 ± 0.11	90 ± 0.13
5.0	92 ± 0.13	98 ± 0.31	93 ± 0.11
10	96 ± 0.21	98 ± 0.11	96 ± 0.09

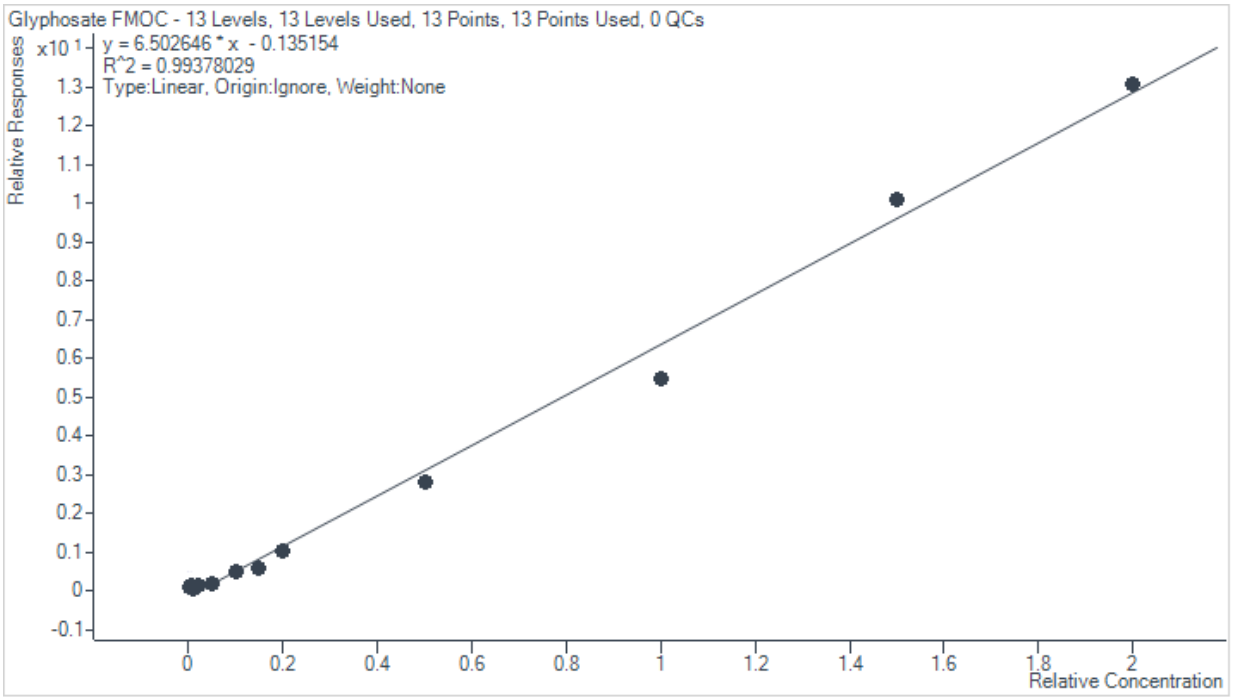


Figure S3.11. Calibration curve for glyphosate.

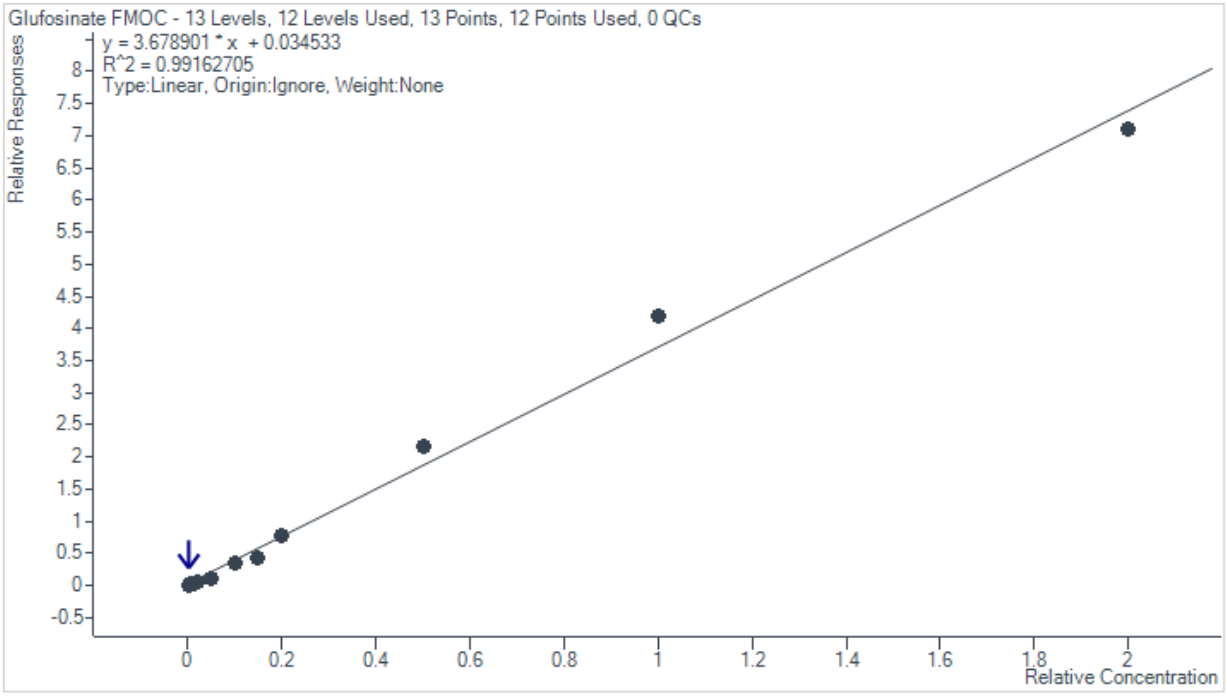


Figure S3.12. Calibration curve for glufosinate.

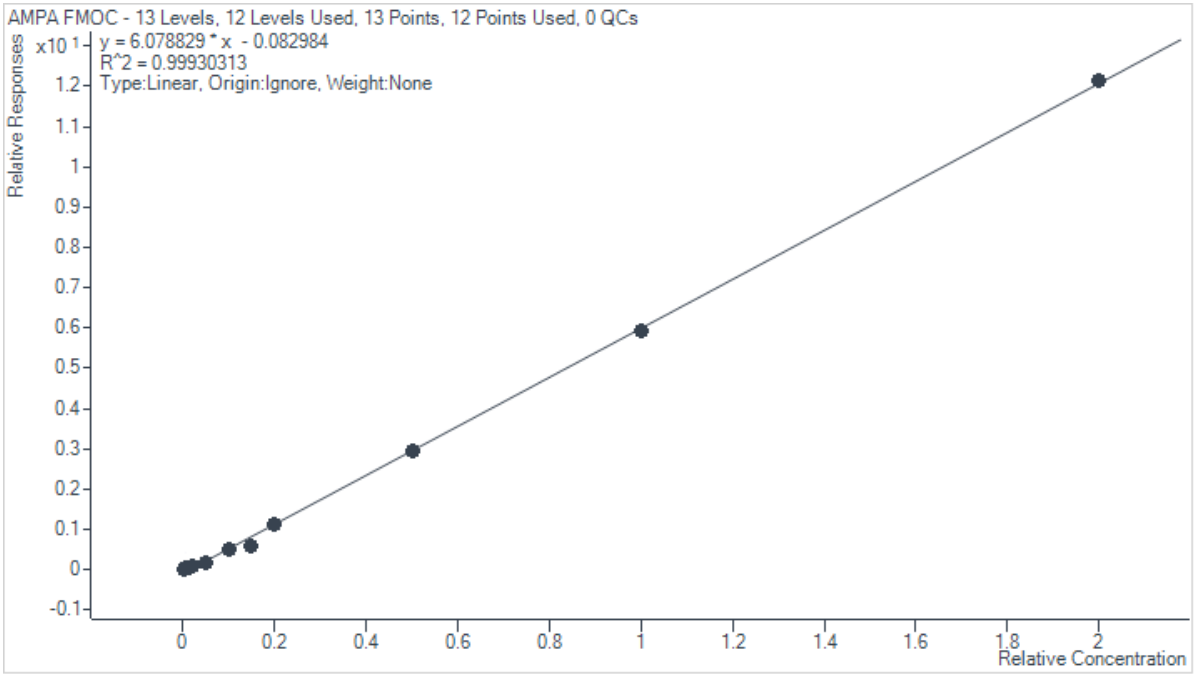


Figure S3.13. Calibration curve for AMPA.

APPENDIX C

Table S4.1. AHP pairwise comparison of Lineament layers.

Pairwise comparisons											
	Class Number	1	2	3	4	5	6	7	8	9	
Class Number	**Layer Description (m^{-1})**	**<1.3**	**1.4 - 3.7**	**3.8 – 5.7**	**5.8 - 7.5**	**7.6 – 9**	**9.1 - 10**	**11 – 12**	**13 – 14**	**15 - 19**	Weight
1	**<1.3**	1	0.5	0.33	0.25	0.2	0.17	0.14	0.13	0.11	2%
2	**1.4 – 3.7**	2	1	0.5	0.33	0.25	0.2	0.17	0.14	0.13	3%
3	**3.8 – 5.7**	3	2	1	0.5	0.33	0.25	0.2	0.17	0.14	4%
4	**5.8 – 7.5**	4	3	2	1	0.5	0.33	0.25	0.2	0.17	6%
5	**7.6 – 9**	5	4	3	2	1	0.5	0.33	0.25	0.2	8%
6	**9.1 - 10**	6	5	4	3	2	1	0.5	0.33	0.25	12%
7	**11 – 12**	7	6	5	4	3	2	1	0.5	0.33	16%
8	**13 – 14**	8	7	6	5	4	3	2	1	0.5	21%
9	**15 - 19**	9	8	7	6	5	4	3	2	1	29%
	CR Value	-0.19									

Lineaments, linear geological features like faults or fractures, can affect pesticide drift and groundwater contamination. They act as preferential pathways for water and contaminants, allowing pesticides to quickly move through and reach groundwater. Pesticides applied near lineaments have higher concentrations in groundwater. To minimize pesticide transport and contamination, the location of these features, using lower-risk pesticides, reduce application rates, and monitor groundwater quality near lineaments is critical.

Table S4.2. AHP pairwise comparison of drainage density layers

Pairwise comparisons											
	Class Number	1	2	3	4	5	6	7	8	9	
Class Number	**Layer Description (m^{-1})**	**<1.5**	**1.6 - 4.4**	**4.5 – 7.1**	**7.2 - 9.3**	**9.3 – 11**	**12 – 14**	**15 - 17**	**18 – 20**	**21 - 27**	Weight
1	**<1.5**	1	0.5	0.33	0.25	0.2	0.17	0.14	0.13	0.11	2%
2	**1.6 – 4.4**	2	1	0.5	0.33	0.25	0.2	0.17	0.14	0.13	3%
3	**4.5 – 7.1**	3	2	1	0.5	0.33	0.25	0.2	0.17	0.14	4%
4	**7.2 – 9.3**	4	3	2	1	0.5	0.33	0.25	0.2	0.17	6%
5	**9.3 – 11**	5	4	3	2	1	0.5	0.33	0.25	0.2	8%
6	**12 – 14**	6	5	4	3	2	1	0.5	0.33	0.25	12%
7	**15 - 17**	7	6	5	4	3	2	1	0.5	0.33	16%
8	**18 – 20**	8	7	6	5	4	3	2	1	0.5	21%
9	**21 - 27**	9	8	7	6	5	4	3	2	1	29%
	CR Value	-0.19									

Drainage density, the number and spacing of drainage channels in a landscape, significantly impacts pesticide drift and groundwater contamination. High drainage density areas, like those with many streams and rivers, are more likely to transport pesticides through runoff, increasing the risk of exposure to aquatic organisms and human populations. Monitoring groundwater quality near high drainage density areas can also help identify potential contamination.

Table S4.3. AHP pairwise comparison of population density layers

Pairwise comparisons											
Class Number	Class Number	1	2	3	4	5	6	7	8	9	Weight
	Layer Description (Population/ sq. km)	**<7300**	**7400-11000**	**12000-14000**	**15000-19000**	**20000-26000**	**27000-38000**	**39000-88000**	**89000-170000**	**180000-310000**	
1	**<7300**	1	0.5	0.33	0.25	0.2	0.17	0.14	0.13	0.11	2%
2	**7400-11000**	2	1	0.5	0.33	0.25	0.2	0.17	0.14	0.13	3%
3	**12000-14000**	3	2	1	0.5	0.33	0.25	0.2	0.17	0.14	4%
4	**15000-19000**	4	3	2	1	0.5	0.33	0.25	0.2	0.17	6%
5	**20000-26000**	5	4	3	2	1	0.5	0.33	0.25	0.2	8%
6	**27000-38000**	6	5	4	3	2	1	0.5	0.33	0.25	12%
7	**39000-88000**	7	6	5	4	3	2	1	0.5	0.33	16%
8	**89000-170000**	8	7	6	5	4	3	2	1	0.5	21%
9	**180000-310000**	9	8	7	6	5	4	3	2	1	29%
	CR Value	-0.19									

Population density significantly impacts pesticide drift and groundwater contamination. High population densities increase the risk of exposure to pesticides, potentially contaminating water sources and air. Urban and suburban areas with impervious surfaces, like concrete and asphalt, also increase the risk of pesticide runoff. Research shows that populations near agricultural

fields have a higher risk of pesticide exposure, with pregnant women in California having higher levels of pesticides in their urine.

Table S4.4. AHP pairwise comparison of infiltration layers

Pairwise comparisons											
	Class Number	1	2	3	4	5	6	7	8	9	Weight
Class Number	**Layer Description**	**Group C/D**	**Group B/D**	**Group A/C**	**Group A/D**	**Group A/B**	**Group D**	**Group C**	**Group B**	**Group A**	
1	**Group C/D**	1	0.5	0.33	0.25	0.2	0.17	0.14	0.13	0.11	2%
2	**Group B/D**	2	1	0.5	0.33	0.25	0.2	0.17	0.14	0.13	3%
3	**Group A/C**	3	2	1	0.5	0.33	0.25	0.2	0.17	0.14	4%
4	**Group A/D**	4	3	2	1	0.5	0.33	0.25	0.2	0.17	6%
5	**Group A/B**	5	4	3	2	1	0.5	0.33	0.25	0.2	8%
6	**Group D**	6	5	4	3	2	1	0.5	0.33	0.25	12%
7	**Group C**	7	6	5	4	3	2	1	0.5	0.33	16%
8	**Group B**	8	7	6	5	4	3	2	1	0.5	21%
9	**Group A**	9	8	7	6	5	4	3	2	1	29%
	CR Value	-0.19									

Infiltration, the rate at which water can penetrate the soil surface, significantly impacts pesticide drift and groundwater contamination. High infiltration rates increase the likelihood of water and pesticides reaching the water table, while low infiltration rates increase the risk of runoff and contamination of nearby water sources. Soil management practices like conservation tillage, cover cropping, and improving soil structure can also help increase infiltration rates and reduce contamination risks. Monitoring groundwater quality in areas with varying infiltration rates can inform management strategies.

Table S4.5. AHP pairwise comparison of precipitation layers

Pairwise comparisons											
	Class Number	1	2	3	4	5	6	7	8	9	Weight
Class Number	**Layer Description (mm)**	**1,107 - 1,151**	**1,152 - 1,171**	**1,172 - 1,192**	**1,193 - 1,212**	**1,213 - 1,232**	**1,233 - 1,255**	**1,256 - 1,279**	**1,280 - 1,311**	**1,312 - 1,379**	
1	**1,107 - 1,151**	1	0.5	0.33	0.25	0.2	0.17	0.14	0.13	0.11	2%
2	**1,152 - 1,171**	2	1	0.5	0.33	0.25	0.2	0.17	0.14	0.13	3%
3	**1,172 - 1,192**	3	2	1	0.5	0.33	0.25	0.2	0.17	0.14	4%
4	**1,193 - 1,212**	4	3	2	1	0.5	0.33	0.25	0.2	0.17	6%
5	**1,213 - 1,232**	5	4	3	2	1	0.5	0.33	0.25	0.2	8%
6	**1,233 - 1,255**	6	5	4	3	2	1	0.5	0.33	0.25	12%
7	**1,256 - 1,279**	7	6	5	4	3	2	1	0.5	0.33	16%
8	**1,280 - 1,311**	8	7	6	5	4	3	2	1	0.5	21%
9	**1,312 - 1,379**	9	8	7	6	5	4	3	2	1	29%
	CR Value	-0.19									

Precipitation significantly impacts pesticide drift and groundwater contamination. The amount and timing of rainfall affect the movement of pesticides through the soil and their potential to reach groundwater. Pesticides can be transported through leaching and runoff mechanisms, with heavy rainfall increasing the risk of leaching and lighter rainfall increasing the risk of runoff. The timing of rainfall also affects the risk of pesticide drift and contamination. Pesticides applied before a rainfall event are more likely to be washed off the soil surface and transported to nearby water sources. During dry weather, pesticides may remain on the soil surface for longer periods, increasing the risk of volatilization.

Table S4.6. AHP pairwise comparison of LULC layers

Pairwise comparisons											
Class Number	Class Number	1	2	3	4	5	6	7	8	9	Weight
	Layer Description	**Barren land**	**Wetland**	**Open Water**	**Mixed forest**	**Herbaceous**	**Pasture**	**Evergreen forest**	**Urban**	**Agriculture**	
1	**Barren land**	1	0.5	0.33	0.25	0.2	0.17	0.14	0.13	0.11	2%
2	**Wetland**	2	1	0.5	0.33	0.25	0.2	0.17	0.14	0.13	3%
3	**Open Water**	3	2	1	0.5	0.33	0.25	0.2	0.17	0.14	4%
4	**Mixed forest**	4	3	2	1	0.5	0.33	0.25	0.2	0.17	6%
5	**Herbaceous**	5	4	3	2	1	0.5	0.33	0.25	0.2	8%
6	**Pasture**	6	5	4	3	2	1	0.5	0.33	0.25	12%
7	**Evergreen forest**	7	6	5	4	3	2	1	0.5	0.33	16%
8	**Urban**	8	7	6	5	4	3	2	1	0.5	21%
9	**Agriculture**	9	8	7	6	5	4	3	2	1	29%
	CR Value	-0.19									

Land use and land cover (LULC) significantly influence pesticide drift and groundwater contamination. Agricultural lands are major sources of pesticide use, which can lead to contamination of water sources. Urban and suburban areas with impervious

surfaces increase the risk of pesticide runoff. LULC also influences the types of pesticides used, with herbicides more frequently used in agricultural areas and insecticides more frequently in residential areas.

Table S4.7. AHP pairwise comparison of thematic layers

Thematic Layers	**Slope (degree)**	**Lineament Density (m^{-1})**	**Drainage Density (m^{-1})**	**LULC**	**Infiltration**	**Precipitation (mm)**	**Population Density (people/km^2)**	**AHP weight (%)**
	Scores							
Slope (degree)	1	1/2	1/3	1/4	1/5	1/6	1/7	**3%**
Lineament Density (m^{-1})	2	1	1/2	1/3	1/4	1/5	1/6	**5%**
Drainage Density (m^{-1})	3	2	1	1/2	1/3	1/4	1/5	**7%**
LULC	4	3	2	1	1/2	1/3	1/4	**11%**
Infiltration	5	4	3	2	1	1/2	1/3	**16%**
Precipitation (mm)	6	5	4	3	2	1	1/2	**24%**
Population Density (people/km^2)	7	6	5	4	3	2	1	**35%**

Table S4.8. USA SSURGO Hydrologic Group

Hydrologic group	Definition
Group A	consist of deep, well drained sands or gravelly sands with high infiltration and low runoff rates.
Group B	consist of deep well drained soils with a moderately fine to moderately coarse texture and a moderate rate of infiltration and runoff.
Group C	consists of soils with a layer that impedes the downward movement of water or fine textured soils and a slow rate of infiltration.
Group D	consists of soils with a very slow infiltration rate and high runoff potential. This group is composed of clays that have a high shrink-swell potential, soils with a high-water table , soils that have a clay pan or clay layer at or near the surface, and soils that are shallow over nearly impervious material.
Group A/D	naturally have a very slow infiltration rate due to a high-water table but will have high infiltration and low runoff rates if drained.
Group B/D	naturally have a very slow infiltration rate due to a high-water table but will have a moderate rate of infiltration and runoff if drained.
Group C/D	naturally have a very slow infiltration rate due to a high-water table but will have a slow rate of infiltration if drained.

Table S4.9. Summary of results from onsite water quality testing with YSI multimeter.

Parameter	Watershed								
	Camden Creek					Cane run and Royal spring			
	SP 1	SP 3/4	SP 7	SP 8	SP 15	PRINT	SPIN	DAIRY	KYHP
Dissolved Oxygen (mg/L)	11±0.02	11±0.03	12±0.02	11±0.01	9.9±0.02	9.7±0.02	12±0.02	14±0.03	14±0.04
Total Dissolved Solids (mg/L)	283±1.3	287±1.9	273±3.3	269±1.6	265±2.1	278±0.5	305±0.5	747±1.3	949±2.8
Nitrate (mg/L)	3.9±0.1	16±0.2	15±0.05	3.2±0.2	4.9±0.1	3.8±0.11	8.7±0.1	12±0.1	4±0.1
Nitrate as Nitrogen (mg/L)	0.87±0.01	3.7±0.03	3.3±0.02	0.73±0.01	1.1±0.01	0.85±0.01	1.9±0.03	2.7±0.03	0.91±0.01
Ammonia (mg/L)	0.83±0.01	0.47±0.01	15±0.04	1.1±0.01	0.23±0.01	0.27±0.01	0.65±0.01	2.9±0.01	3.7±0.01
Ammonia as Nitrogen (mg/L)	0.65±0.01	0.37±0.01	12±0.09	0.81±0.01	0.18±0.01	0.21±0.01	0.51±0.01	2.3±0.02	2.9±0.05
Salinity (psu)	0.21±0.01	0.21±0.03	0.23±0.03	0.18±0.01	0.08±0.01	0.18±0.01	0.25±0.03	0.57±0.03	0.73±0.03
pH	7.1±0.5	6.8±0.06	7.9±0.12	7.1±0.12	7.3±0.17	6.9±0.22	7.9±0.32	7.9±0.19	7.4±0.18
Turbidity (FNU)	16±0.12	3.3±0.03	54±0.93	2.2±0.03	0.87±0.03	3.2±0.03	12±0.53	14±0.23	2.5±0.03

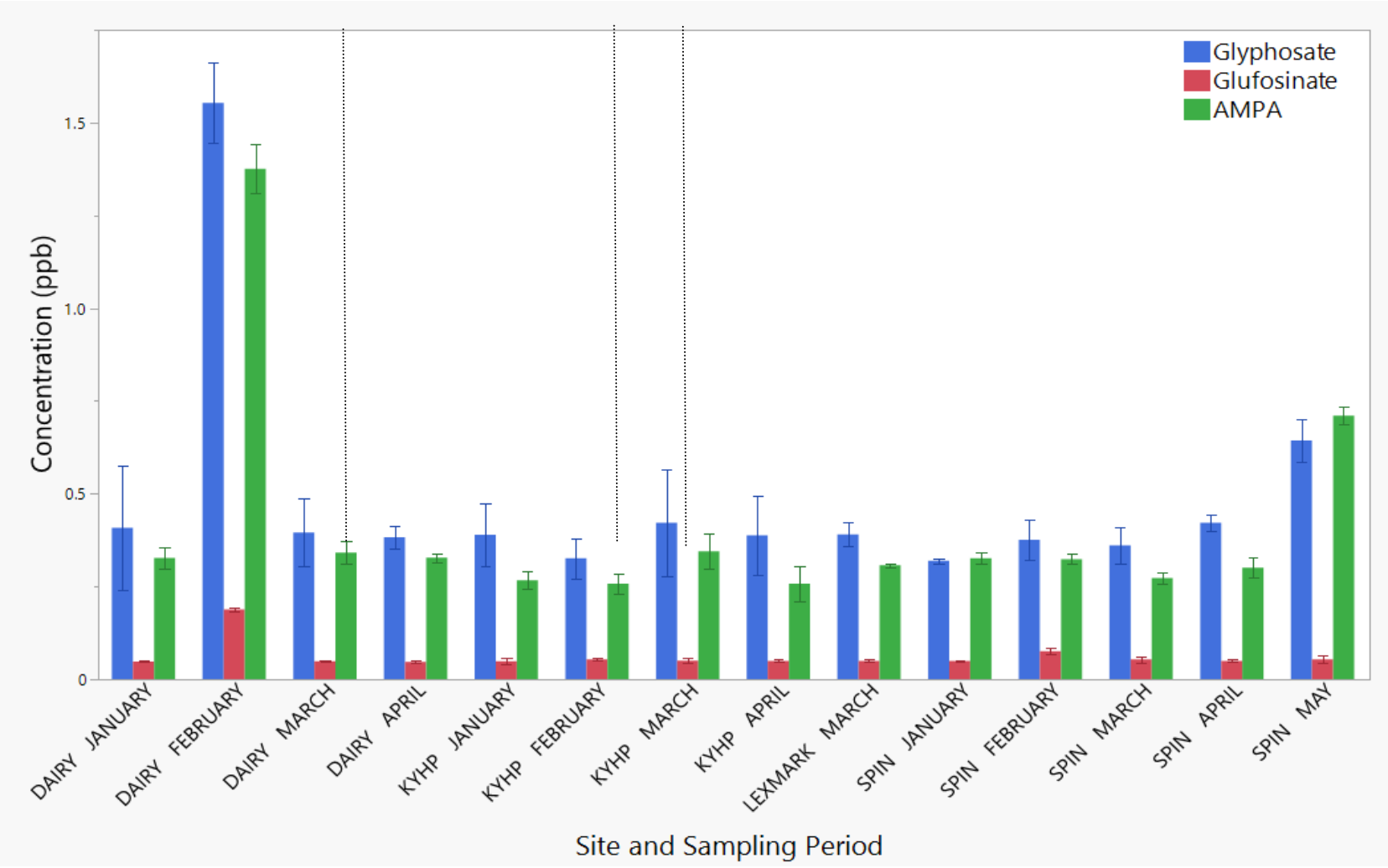


Figure S4.1. Detected concentration of glyphosate, glufosinate and AMPA from January 2022 to June 2022 in the Cane run and royal spring watershed.

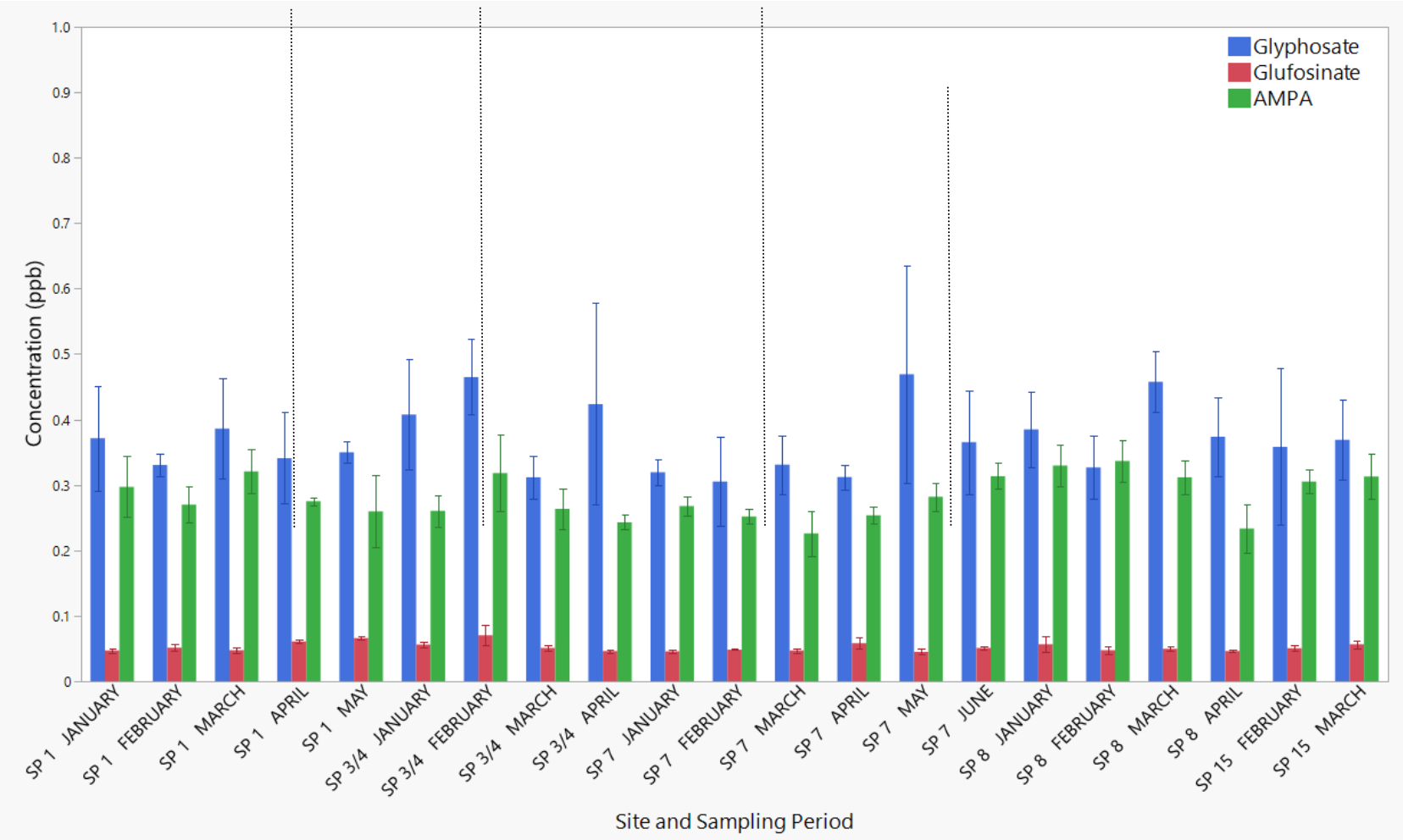


Figure S4.2. Detected concentration of glyphosate, glufosinate and AMPA from January 2022 to June 2022 in the Cane run and royal spring watershed.

APPENDIX D

Table S5.1: Dunnett's Test of the influence of various thematic layers.

Omission of thematic layer	**p-Value**
No Population density	< 0.05
No Precipitation	< 0.05
No LULC	< 0.05
No Drainage density	0.1
No Slope	0.1
No Infiltration	0.5
No Lineament density	0.9

Table S5.2. Pairwise T-test of simulated thematic layer scenarios.

	GVI Classification (%)				
	Very Low	**Low**	**Prone**	**High**	**Very High**
Original GVI	7	24	31	24	14
Scenario 1 vs Scenario 2	4	16	30	29	21
Scenario 1 vs Scenario 3	6	20	28	30	16
Scenario 1 vs Scenario 4	3	24	28	26	19
Scenario 1 vs Scenario 5	5	22	27	26	20
Scenario 2 vs Scenario 4	7	24	25	25	19
Scenario 2 vs Scenario 5	7	24	23	25	21
Scenario 3 vs Scenario 4	8	24	30	24	14
Scenario 3 vs Scenario 5	10	22	30	24	14
Scenario 4 vs Scenario 5	9	24	29	24	14

Table S5.3. ANOVA results of full factorial evaluation of various scenarios at 2% population density increment.

Third scenario factorial evaluation			**p-value**
Scenario 1	Scenario 2	Scenario 4	< 0.05
Scenario 1	Scenario 2	Scenario 5	< 0.05
Scenario 1	Scenario 3	Scenario 4	< 0.05
Scenario 1	Scenario 3	Scenario 5	< 0.05
Scenario 1	Scenario 4	Scenario 5	< 0.05
Four scenario factorial evaluation			**p-value**
Scenario 1	Scenario 2	Scenario 4	Scenario 5	< 0.05
Scenario 1	Scenario 3	Scenario 4	Scenario 5	< 0.05