Relevance of the Antiscalant DTPMP for Surface Water Contamination with Glyphosate and AMPA

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Ich erkläre hiermit, dass ich die zur Promotion eingereichte Arbeit mit dem Titel: "Relevance of the antiscalant DTPMP for the surface water contamination of glyphosate and AMPA" selbständig verfasst und nur die angegebenen Quellen und Hilfsmittel benutzt und wörtlich oder inhaltlich übernommene Stellen als solche gekennzeichnet habe. Ich erkläre, dass die Richtlinien zur Sicherung guter wissenschaftlicher Praxis der Universität Tübingen (Beschluss des Senats vom 25.5.2000) beachtet wurden. Ich versichere an Eides statt, dass diese Angaben wahr sind und dass ich nichts verschwiegen habe. Mir ist bekannt, dass die falsche Angabe einer Versicherung an Eides statt mit Freiheitsstrafe bis zu drei Jahren oder mit Geldstrafe bestraft wird.

Tübingen, den 01.03.2025

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Lisa Maria Engelbart

Table of Contents

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Author contribution

Erklärung nach §6 Abs. 2 der Promotionsordnung der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Tübingen

Prof. Carolin Huhn supervised my thesis, supported me with scientific ideas and interpretations and also revised the written thesis. Furthermore, my work for this thesis was supported by other researchers and interns in the laboratory work, in the interpretation of the data and in the revision but also in sampling campaigns. A transparent disclosure of author contributions is provided in the following.

Motivation, Summary and outlook

This part was written by me and revised by Prof. Carolin Huhn.

Section 2.1: Glyphosate contamination in European rivers not from herbicide application*?*

The hypothesis for this chapter was developed by Prof. Carolin Huhn inspired by sediment core data from Benedikt Wimmer. Data collections and investigation regarding sampling sites were carried out by Prof. Carolin Huhn and Marc Schwientek. Main parts of this manuscript were written by her. Dr. Marc Schwientek contributed to data interpretation, supported by Dr. Hermann Rügner. Dr. Wolfgang Schulz supported this work with data organization and his expertise on wastewater markers. Prof. Stefan Haderlein assisted by critically reviewing the results presented. Sarah Bieger and I conducted literature search and aided in the preparation and interpretation of figures. All aforementioned authors revised the manuscript and took part in discussions of its content. This section was published: Schwientek, H., Rügner, S. B., Haderlein, S. B., Schulz, W., Wimmer, B., Engelbart, L., Bieger, S., & Huhn, C. (2024). Glyphosate contamination in European rivers not from herbicide application? Water Research, 263, 122140. 10.1016/j.watres.2024.122140

Section 2.2: This section summarizes separation techniques of the last 40 years for the analysis of amino(poly)phosphonates.

Literature research and writing was conducted by myself. My supervisor, Prof. Carolin Huhn, aided me in the structuring and revision of the content and text.

Section 3: New approaches for enrichment and analysis of aminopolyphosphonates and their transformation products

Practical work was partly conducted by my interns Mostafa Nowroozi and Noah Buth under my supervision. Mostafa Nowroozi performed first enrichment experiments and Noah Buth conducted some CE-MS and LC-MS measurements for method development. Further LC-MS measurements were performed at the Leibniz Institute for Tropospheric Research in Leipzig with the support of Christina Breitenstein in the laboratories of Prof. Hartmut Herrmann. Results were discussed with Prof. Carolin Huhn, who also revised this section.

Section 4: In-situ formation of glyphosate and AMPA in sewage sludge from phosphonates used as antiscalants and bleach stabilizers in households and industry

This section is a modified version of the manuscript submitted for publication in Water Research. Prof. Carolin Huhn was the lead supervisor of this study. The manuscript in this section was written jointly by Prof. Carolin Huhn, Sarah Bieger and myself. Additional information was added to the submitted manuscript by me. Sarah Bieger and I conducted all experiments and data evaluation and prepared all figures equally. Dr. Katharine Thompson designed the first experiment with activated sludge from Büsnau and aided in discussing the obtained results. Hermann Rügner supported sediment and activated sludge sampling. During his internship, Leon Fischer synthesized 13C5-labeled DTPMP, supervised by Sarah Bieger. Some aqueous samples were analyzed for glyphosate and AMPA by LC-MS at the Zweckverband Landeswasserversorgung Langenau thanks to Tobias Bader. NMR analysis was supported by Markus Kramer. Background information and deferrization sludge sample were provided by Robert Bloch from the Berliner Wasserbetriebe. Prof. Stefan Haderlein, Anna Röhnelt, Dr. Philipp Martin and Dr. Daniel Buchner discussed the experiments and results. All aforementioned authors took part in the revision and proof-reading of the submitted manuscript. Employees of the wastewater treatment plants Pliezhausen Rübgarten and Tübingen kindly provided information about the wastewater treatment plants and samples.

Section 5: Relevance of a wastewater treatment plant on the spatial variability of glyphosate, AMPA and DTPMP in the receiving river before and after its closure

Prof. Christiane Zarfel made the research of this section possible by establishing contact with Dr. Jonas Scharper, who was in charge for the sampling campaign and provided all samples. Practical work was partly conducted by my interns Philipp Steinbuch and Clemens Wenzel, who aided in sample preparation for sediment samples analysis. Aqueous samples were analyzed for glyphosate and AMPA by LC-MS at the Zweckverband Landeswasserversorgung Langenau thanks to Markus Flörs. Sarah Bieger supported this work regarding practical aspects such as sample transport/preparation and measurements, but also by discussing obtained results with me and my supervisor, Prof. Carolin Huhn. Jennifer Kaupp conducted experiments for the extraction efficiency of DTPMP under my supervision. Jonas Schaper also provided carbamazepine results shown in this section and supported this work with his expertise. I prepared the first draft and Prof. Carolin Huhn and Dr. Jonas Schaper revised this part of my thesis.

Abbreviation

A110, AMPA	Aminomethylphosphonic acid
A112	AMPA-^{13}C-^{15}N
A114	AMPA-^{13}C-^{15}N-methylene-D_2
AC	Abiotic control
ACN	Acetonitrile
AM	Analyte mixture
APP	Aminopolyphosphonat
ATMP	Aminotris(methylene phosphonic acid)
BC	Biotic control
BGE	Background electrolyte
CE	Capillary electrophoresis
DH	Diluted mineral suspension shaken horizontal
DOC	Dissolved organic carbon
DTPMP	Diethylenetriaminepenta(methylene phosphonic acid)
DV	Diluted mineral suspension shaken vertical
EABMP	Ethanolamine bis (methylene phosphonic acid)
EC	Electrical conductivity
EDTA	Ethylenediaminetetraacetic acid
EDTMP	Ethylenediamine tetra(methylene phosphonic acid)
EOF	Electroosmotic flow
EQS	Environmental quality standard
FMOC-Cl	Fluorenylmethyloxycarbonyl chloride
G168	Glyphosate
G169	Glyphosate-2-^{13}C
G170	Glyphosate-2-^{13}C-^{15}N
G172	Glyphosate-$^{13}C_3$-^{15}N
HDTMP	Hexamethylenediamine tetra(methylene phosphonic acid)
HILIC	Hydrophilic interaction liquid chromatography
IC	Ion chromatography
IDMP	Iminodi (methylphosphonic acid)
IE	Inhabitant equivalent
IPD	Indirect photometric detection
IRMS	Isotope-ratio mass spectrometry
LOD	Limit of detection

LOQ	Limit of quantification
LW	Low water content mineral suspension
PAD	Pulsed amperometric detection
PB-MS	Particle beam-mass spectrometry
PBTC	2-phosphonobutane 1,2,4-tricarboxylic acid
pH_{pzc}	pH point of zero charge
RPLC	Reversed phase liquid chromatography
SPE	Solid phase extraction
WWTP	Wastewater treatment plant

Zusammenfassung

Das Herbizid Glyphosat und Aminomethylphosphonsäure (AMPA), ein Transformationsprodukt von Glyphosat und anderen Aminopolyphosphonaten, sind weit verbreitete Schadstoffe in europäischen Oberflächengewässern. Es wurde bereits gezeigt, dass Kläranlagen eine der Hauptquellen für die Emission von Amino(poly)phosphonaten sind. Die relativ konstanten Glyphosat- und AMPA-Massenflüsse in Vorflutern von Kläranlagen während des ganzen Jahres deuten auf andere Quellen für diese Stoffe als die nicht-urbane Verwendung des Herbizids hin, was zur Hypothese führte, dass Glyphosat ein Abbauprodukt von Aminopolyphosphonaten ist. Aufgrund der komplexen Analytik von Aminopolyphosphonaten gibt es aber nur wenige Studien zu ihrem Abbau oder Vorkommen in der Umwelt. Um dies zu erleichtern habe ich Ansätze für derivatisierungsfreie Trennmethoden mittels Kapillarelektrophorese-Massenspektrometrie (CE-MS) und hydrophile Interaktionschromatographie-Massenspektrometrie (HILIC-MS) für die Analyse von großen und kleinen Amino(poly)phosphonaten erarbeitet. Um die Analyse von Glyphosat ebenfalls in Oberflächengewässern mittels CE-MS zu ermöglichen, habe ich verschiedene Bodenminerale zur Anreicherung und Extraktionsmedien zur Desorption getestet um die Nachweisgrenzen zu verbessern. Des Weiteren gelang erstmals der experimentelle Nachweis der Bildung von Glyphosat und AMPA aus DTPMP in Belebtschlamm und damit der Nachweis einer neuen, bisher nicht beachteten Quelle für Glyphosat. Zur Bewertung der Umweltrelevanz meiner Befunde habe ich den Vorfluter einer Kläranlage in der Nähe von Nürnberg während ihres Betriebs und nach ihrer Abschaltung untersucht. Der Kläranlagenabfluss konnte eindeutig als wichtigste Quelle für DTPMP, Glyphosat und AMPA identifiziert werden. Die Abnahme der Konzentrationen entlang des Flussverlaufs deutet auf verschiedene Effekte wie Abbau, Bildung, Verdünnung und Sorption hin. Die Abschaltung der Kläranlage führte im Vorfluter zu einer schnellen Abnahme der Konzentrationen von Glyphosat, AMPA und DTPMP im Wasser und Sediment des Vorfluters. Dies zeigte, dass sich auch ein stark belasteter Fluss innerhalb eines Jahres in Bezug auf die betrachteten Kontaminanten erholen kann.

Insgesamt zeigt diese Arbeit die in-situ Bildung von Glyphosat und AMPA aus DTPMP in Belebtschlamm, neue Ansätze für die Analytik von Amino(poly)phosphonaten und den Einfluss einer Kläranlagenabschaltung auf die Belastung des Vorfluters mit Glyphosat, AMPA und DTPMP.

Abstract

The herbicide glyphosate and aminomethylphosphonic acid (AMPA), a transformation product of glyphosate and other aminopolyphosphonates, are widespread pollutants in European surface waters. Wastewater treatment plants (WWTPs) have been identified as a major source of amino(poly)phosphonate emission. However, rather constant glyphosate and AMPA mass fluxes in receiving rivers throughout the year indicate other sources than urban herbicide application. For this reason, glyphosate was hypothesized to be a transformation product of aminopolyphosphonates. Due to analytical constraints, environmental findings regarding aminopolyphosphonates are scarce. In my thesis, I developed approaches for derivatization-free analytical methods to separate both large and small amino(poly)phosphonates using capillary electrophoresis-mass spectrometry (CE-MS) and hydrophilic interaction chromatography-mass spectrometry (HILIC-MS) for environmental sample analysis. Furthermore, I investigated different soil minerals in combination with extraction media for the enrichment of glyphosate from surface water to enable its analysis by CE-MS and improve limits of detection. Additionally, I demonstrated experimentally that glyphosate and AMPA formation from DTPMP in activated sludge is possible, providing an additional, previously neglected source of glyphosate. To assess the environmental relevance of my findings, I investigated the receiving river of a WWTP near Nuremburg during operation and after its shutdown. I identified the WWTP effluent as the dominant source of DTPMP, glyphosate and AMPA contamination. A decrease in concentrations along the river stretch indicated different attenuation processes such as (trans)formation, dilution and sorption. After ~~the~~ WWTP shutdown, glyphosate, AMPA and DTPMP concentrations rapidly decreased in both surface water and sediment of the previously highly contaminated receiving river, demonstrating the river´s fast recovery after WWTP shutdown. Overall, in this thesis, I demonstrated the in-situ formation of glyphosate and AMPA from DTPMP in activated sludge, presented new approaches for amino(poly)phosphonate analysis, highlighted the significant impact of WWTPs and demonstrated the improvement of the chemical status of the river regarding glyphosate, AMPA and DTPMP concentrations after their source was eliminated with the shutdown of the WWTP.

1 Motivation

The use of the herbicide glyphosate is intensely debated given its high global consumption. Nevertheless, its use was re-approved in the EU in 2023 until 2033.[1] In Germany, glyphosate use is already strongly regulated, with only small application windows allowed for agricultural use and prohibition on its use e.g. for siccation and private use.[2] The Deutsche Bahn with its previously intense use of glyphosate for weed control along railway tracks stated the renouncement of glyphosate application for train track maintenance by 2023 and has already reduced its use from 2018 to 2020 by 50%,[3] based on a consumption of 65.4 tons in 2017.[4] Studies from around the globe showed widespread contamination of surface waters with both glyphosate and its main transformation product aminomethylphosphonic acid (AMPA).[5-12] Agriculture is commonly regarded as the main emission source, however, several studies demonstrated wastewater treatment plants (WWTPs) as an important source for glyphosate.[5, 9, 12-14] Schwientek et al. recently discussed wastewater even as the dominant source of glyphosate in Europe based on an evaluation of long-term concentration patterns in rivers.[5] The authors demonstrated that most European surface waters have rather constant mass fluxes of glyphosate throughout the year, otherwise only known for e.g. pharmaceuticals and household chemicals. The presence of an alternative source of glyphosate and AMPA than urban herbicide use was discussed and aminopolyphosphonates were suspected to form glyphosate and AMPA in-situ in the sewer systems and WWTPs.[5] From sales numbers and its chemical relationship to glyphosate, diethylenetriaminepenta(methylene-phosphonic acid) (DTPMP) was in the focus. In general, phosphonates are used in high volumes in Europe, with a total phosphonate consumption of 10,000-50,000 tons,[15] and enter wastewater treatments plants e.g. by their application in laundry detergents from households. The formation of glyphosate by ozonation of ethylenediamine tetra(methylene phosphonic acid) (EDTMP) [16] and from DTPMP in presence of MnO_2 [17] have already been demonstrated. In WWTPs, phosphonates were discussed to be eliminated with rates of up to 80-95%[18], mainly due to adsorption to activated sludge.[19-21]

This work aims to contribute to a better understanding of the fate and occurrence of not only DTPMP, where little work has been published so far [22, 23] but also its transformation products in environmental samples and a possible in-situ formation of glyphosate and AMPA in activated sludge. DTPMP was persistent in transformation experiments based on the OECD 302B test guideline recording CO_2 formation.[24] So, the processes relevant in WWTPs need to be elucidated also based on a discussion of possible transformation pathways. All these questions in environmental chemistry can only be answered when new methods are developed capable of analyzing DTPMP and its transformation products, among them glyphosate and AMPA in diverse environmental matrices including sediment, wastewater and activated sludge.

2 Introduction

2.1 State of the art of separation techniques concerning aminopolyphosphonates in environmental samples

2.1.1 Abstract

Only a few separation methods have been published for the analysis of aminopolyphosphonates (APPs), which are summarized in this review. We here focus on the major APPs in use, which include aminotris(methylene phosphonic acid) (ATMP), ethylenediamine tetra(methylene phosphonic acid) (EDTMP) and diethylenetriamine penta(methylene phosphonic acid) (DTPMP) and some of their transformation products, e.g. iminodi(methylene phosphonic acid)(IDMP). The emphasis is on analytical methods applicable to environmental samples to demonstrate their suitability for monitoring surface water, sediments, and wastewater. Chromatographic and electrophoretic separation were used with various detection techniques including mass spectrometry. Limits of detection were reached in the two-digit µg/L range by using ion chromatography (IC) with pulsed amperometric detection and <0.2 µg/L when using IC-(ESI-)MS. These methods were applied for the analysis of various environmental samples such as wastewater influent/effluent and sediment extracts. Separation by hydrophilic interaction liquid chromatography (HILIC) coupled to ESI-MS seems promising, but at the moment its applicability to extracts of sediment or sewage sludge has not yet been demonstrated. Several studies used derivatization techniques to make the phosphonates amenable to analysis by reversed phase liquid chromatography (RPLC)-ESI-MS, e.g. with a methylation of the phosphonic acid groups. Separation using capillary electrophoresis (CE) is also reported, but not yet reaching environmentally relevant concentrations. For APP analysis, elevated sample preparation techniques were mostly applied to reduce matrix effects e.g. to reduce the amount of metal cations interfering separation by using anion exchange resins. Clearly, simplified sample preparation schemes would be advantageous to facilitate monitoring strategies. For IC and CE separations, sample preparation may be reduced as a direct analysis without derivatization is possible. Currently, no standard method for analysis of APPs is available. This also holds true for the analysis of their transformation products, which were hardly addressed in environmental samples. Therefore, the knowledge on their occurrence and relevance in the environment is insufficient.

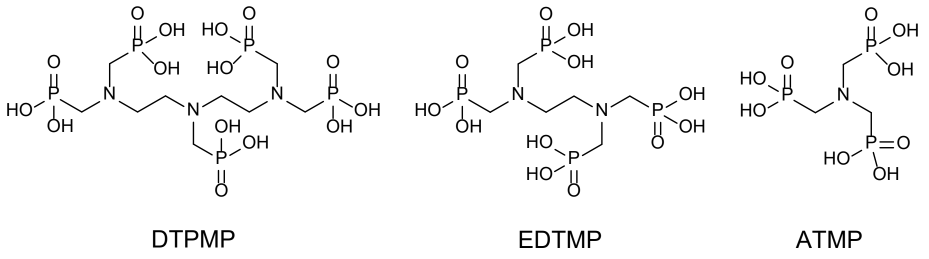
2.1.2 Introduction

Aminopolyphosphonates (APPs) are high-volume anthropogenic compounds widely used as powerful chelating agents in household products and various industrial applications. In household applications, they are used in detergents and cleaning products,[25] particularly as bleach stabilizers and anti-scaling agents. Industrially, APPs are used in cleaning processes, e.g. in the food industry, in membrane treatment for reverse osmosis in drinking water production [26-29] and desalination,[30] and in the prevention of scale and biofouling in cooling water systems.[31] They also act as plasticizers in concrete [32] and are used in the paper, metal and textile industries.[18] In Europe alone, approximately 12,000 tons of phosphonates were used in household products in the early 2010s,[15] contributing to a total European consumption of 49,000 tons in 2013.[18] The commercially most relevant APPs include diethylenetriamine penta(methylene phosphonic acid) (DTPMP), aminotris(methylene phosphonic acid) (ATMP), and, to a lesser extent, ethylenediamine tetra(methylene phosphonic acid) (EDTMP). Their chemical structures are shown in Fig. 1.

Despite their widespread use, little is known about their occurrence in the environment. A major limitation is the lack of commercially available analytical standards with sufficient purity (including isotope-labeled standards) for these compounds compared to e.g. pesticides. This holds true also for most of their transformation products. This further complicates the development of new analytical methods including sample preparation and especially quantification, as technical mixtures often contain impurities minor substituted with methylene phosphonate moieties than the nominal compound.[33]

APP properties that are advantageous in their applications, such as complexation [34] and strong sorption,[16, 35] often complicate the analysis of these substances. They are very polar and have functional groups charged at most environmentally relevant pH values,[36] have no chromophore, are non-volatile and highly soluble in water but not in organic solvents. The combination of all these analytical constraints have significantly hindered the availability of analytical methods suitable to study their environmental fate and transformation pathways. Currently, there is one more recent study with a broader scope, which determined the phosphonates ATMP, DTPMP and EDTMP in environmental samples including wastewater treatment plant (WWTP) influent (<LOD-71 µg/L), wastewater solids (<LOD-312 mg/kg), river water (<LOD-8 µg/L) and receiving water sediments (<LOD-5 mg/kg).[22] Jaworska et al. predicted APP concentrations between 0.04-1.38 µg/L in surface water and 0.003-0.300 mg/kg in sediment for the Netherlands.[23]

Transformation was mainly investigated analyzing mineralization products such as orthophosphate [37] or CO_2.[24] However, these approaches provide only a partial understanding of the transformation processes, as intermediate transformation products are not covered but may be stable or even persistent. Only aminomethylphosphonic acid (AMPA) and sometimes iminodi(methylene phosphonic acid) (IDMP) were monitored additionally,[37, 38] with AMPA known to be persistent with half-lives of 12 days in soils [39] and 52.5 days in water.[40] While some insight into ATMP transformation products in WWTPs was reported,[38] studies on the transformation products of other APPs and their occurrence in the environment remains scarce.[41-43] A recent study highlighted the in-situ formation of glyphosate formed from DTPMP in laboratory experiments with aqueous model solutions [17] and in incubation experiments using activated sludge [44] – still, the complete transformation pathways of e.g. DTPMP and EDTMP are far from being fully understood as the large number of possible transformation products from C-N and C-P cleavage and oxidation cannot be covered by analytical techniques currently available.



DTPMP

EDTMP

ATMP

Fig. 1: Structures of the aminopolyphosphonates (free acids) with highest sales numbers: DTPMP: diethylenetriamine penta(methylene phosphonic acid), EDTMP: ethylenediamine tetra(methylene phosphonic acid), ATMP: aminotris(methylene phosphonic acid).

All these aspects highlight the need for powerful analytical methods capable of handling complex environmental matrices. This review aims to provide an overview of the current state of APP analysis, focusing on the major APPs ATMP, DTPMP and EDTMP, highlighting achievements in the field and identifying major aspects for improvement. By addressing existing analytical gaps, this study aims to pave the way for a more effective environmental monitoring enabling a deeper understanding of the fate and occurrence of APPs in the environment.

2.1.3 Results and Discussion

In the following, different analytical methods used for the analysis of the main APPs ATMP, EDTMP and DTPMP and some of their transformation products such as IDMP and ethanolamine bis (methylene phosphonic acid) (EABMP) are elaborated. Table 1 summarizes all published separation methods for APPs and some transformation products included in this review. We discriminate between methods with and without MS detection, as both approaches are of interest in different fields of application. Methods using detection techniques other than MS are more cost-effective and can be a great tool e.g. for purity control of commercial products and in e.g. kinetic or sorption studies in model samples. In contrast, MS detection, especially high-resolution MS, is required to identify transformation pathways, enable non-target screening approaches and identify analytes even when baseline separation is not achieved. Furthermore, identification of the many different expected transformation products solely relying on the retention time is not possible due to lack of commercial standards as references. Finally, different sample preparation techniques and their application to environmental samples are discussed as no analytical standard method is available yet.[18]

2.1.3.1 Separation methods for the analysis of ATMP, EDTMP, DTPMP and IDMP

A) without MS detection: Historically, the first methods used to analyze APPs did not use MS for detection as many methods were published in the 1980s and 1990s. Nevertheless, a number of methods were available, mostly using ion chromatography (IC) for the separation of the charged APPs. As the analytes themselves have no chromophore, some methods used complexes with Fe(III) ions [45-47] or vanadate-molybdate [48] to enable UV-detection. In these studies, detection limits of 15-57 µg/L were reached in natural waters,[47] but matrix effects from competitive complexation can be expected in more complex environmental samples such as sediment extracts. Furthermore, detection methods based on Fe(III)-complexation were reported to suffer from a high background noise due to the necessary mixing of the reagents with the eluent.[49] The most recent IC method developed for the analysis of ATMP, IDMP, EDTMP and DTPMP uses pulsed amperometric detection (IC-PAD) (exemplary chromatogram see Fig. 2A).[50] According to the Analytical GREEnness metric system by Pena-Pereira et al.[51] the method better follows the twelve principles of green analytical chemistry compared to an IC-ESI-MS [52] or IC-UV method,[47] but LODs were relatively high with 9-60 µg/L and did not improve earlier methods with reported for UV detection of metal complexes (LODs of 15-57 µg/L).[47]

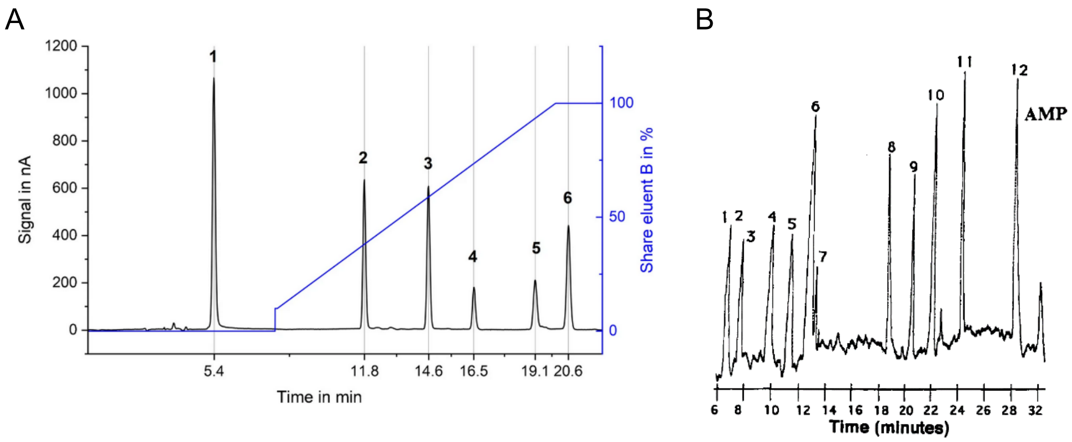


Fig. 2: (A) Chromatogram of the optimized separation using IC with pulsed amperometric detection of a multi-phosphonate standard (1-2 mg/L AMPA (1), glyphosate (2) and IDMP (3), 0.3 mg/L ATMP (4), 4 mg/L EDTMP (5) and 6 mg/L DTPMP (6) with an injection volume of 50 µL). Data treatment included blank subtraction. Source: Röhnelt et al., Anal Bioanal Chem (2025), CC BY 4.0.[50] (B) Electropherogram of a phosphonate mixture using CE with indirect photometric detection. Annotation (selected): Peaks 10 = ATMP, 11 = EDTMP, 12 = DTPMP at concentrations of ca. 10 mg/L. Reprinted (adapted) with permission from Shamsi and Danielson, Anal. Chem. 1995, 67, 11, 1845–1852, Copyright 1995, American Chemical Society.[53]

Using capillary electrophoresis (CE) separation, a UV-absorbing background electrolyte was chosen to detect analytes by indirect photometric detection (exemplary electropherogram see Fig. 2B),[53] but LODs were high with >1,000 µg/L. This may be sufficient for the characterization of technical products, but far too high for environmental samples, which are currently reported to be mostly far below <100 µg/L.[22, 23] Methods using conductivity detection have also been successfully used for the detection of phosphonates,[54, 55] but since the detection limit depends on the difference in conductivity between background electrolyte (BGE) co-ion and analyte, improvements of the LOD may simultaneously reduce separation efficiencies due to a mismatch of electrophoretic mobilities of analytes and ions in the BGE.

In the methods discussed in this section, identification of analytes is only reached via retention or migration times using commercial standards, but no further identification of transformation products is possible.

We regard methods such as IC-PAD [50] to be a useful tool, e.g. for the quality control of technical products, being cost-efficient and having low requirements for toxic chemicals. But they are not suitable for the analysis of phosphonates and (unknown) transformation products in environmental samples with elevated matrix loads or samples from transformation experiments as (unknown) transformation products cannot be identified or quantified.

Table 1: Overview of IC, CE and RPLC methods using different detection for the analysis of the main aminopolyphosphonates ATMP, EDTMP, DTPMP and transformation products IDMP, EABMP[d], glyphosate and AMPA including the stationary phase or capillary used for separation, the eluents or the background electrolyte (BGE) and APPs included in the study and reference. LOD/LOQ are stated if available.

Separation	Detection	Column/ capillary	Separation medium	Analytes	Remarks (derivatization,s heath liquid)	LOD/ LOQ/ µg/L	Ref.
IC	conductivity	AG11	30 mM Na_2CO_3	IDMP methyl-IDMP formyl-IDMP			[54]
	conductivity	IonPac AG16	A: 20 mM NaOH B: 65 mM NaOH	ATMP			[55]
	PAD[a]	Metrosep Carb 2	A: 345 mM sodium acetate B: 15 mM NaOH	AMPA IDMP			[56]
	PAD[a]	IonPac AS16 RFIC, MFC500	A: 15 mM NaOH B: 15 mM NaOH + 400 mM sodium acetate	AMPA glyphosate IDMP ATMP EDTMP DTPMP		1.55-59.62	[50]
	UV 260 nm	PLRP-S	A: water, 0.02 M sodium bicarbonate, 0.001 M tetrabutylammoniumbromide B: 14% sodium bicarbonate in ACN	ATMP EDTMP DTPMP	Fe(III) complexes	14.95-57.32	[47]
	UV 300 nm	Dionex HPIC AS7/AG7	15 mM HNO_3	EDTMP DTPMP	Fe(III) complexes		[45]
	UV 330 nm	Dionex HPIC-AS7	0.03 mM HNO_3	ATMP EDTMP DTPMP	Vanadate-molybdate complexes		[46]
	UV 410 nm	Dionex HPIC-AS7	0.2 mM ethylenediaminetetraacetic acid + 0.17-0.5 M KCl, pH 5.1	ATMP EDTMP DTPMP HDTMP[b]	Fe(III) complexes		[48]

[a]PAD: Pulsed amperometric detection, [b]HDTMP: Hexamethylenediamine tetra(methylene phosphonic acid), [c]IRMS: Isotope-ratio mass spectrometry, [d]EABMP: Ethylaminobis(methylenephosphonic) acid, [e]published without addition of formic acid, otherwise same separation conditions, [f]PB-MS: Particle beam - mass spectrometry, [g]IPD: Indirect photometric detection

Separation	Detection	Column/ capillary	Separation medium	Analytes	Remarks (derivatization,s heath liquid)	LOD/ *LOQ*/ µg/L	Ref.
IC	ESI-MS	IonPac AS16	A: 20% MeOH/ 80% H_2O B: 200 mM NaOH, 20% MeOH/ 80% H_2O	ATMP EDTMP DTPMP		0.04-0.16	[52]
	ICP-MS	IonPac AS16	A: H_2O + 300 µg/L diethylenetriaminepentaacetic acid B: NaOH + 300 µg/L diethylenetriaminepentaacetic acid	ATMP EDTMP DTPMP		0.002-0.025 0.018-0.116	[57]
	IRMS[c]	IC NI-424	4 mM sulfuric acid	ATMP EDTMP			[58]
	ESI-MS	RP-C18	A: H_2O B: ACN	ATMP EDTMP DTPMP	tetraethyl-ammonium acetate		[59]
	ICP-MS	microbore AS11, online metal removal	A: H_2O B: 200 mM ammonium nitrate	EDTMP			[60]
HILIC	ESI-MS	LUNA HILIC	A: 100% H_2O + sodium acetate B: 10% H_2O /90% ACN + 2.5 mM sodium acetate	IDMP ATMP EDTMP DTPMP			[61, 62]
	ESI-MS	SeQuant ZIC-HilicHILIC	A: 100% H_2O 100 mM ammonium formate B: 10% H_2O /90% ACN 10 mM ammonium formate	IDMP EABMP[d] EDTMP[e] DTPMP			[42, 63]

[a]PAD = Pulsed amperometric detection, [b]HDTMP = Hexamethylenediamine tetra(methylene phosphonic acid), [c]IRMS = Isotope-ratio mass spectrometry, [d]EABMP = Ethylaminobis(methylenephosphonic) acid, [e]published without addition of formic acid, otherwise same separation conditions, [f]PB-MS = Particle beam - mass spectrometry, [g]IPD = Indirect photometric detection

Separation	Detection	Column/ capillary	Separation medium	Analytes	Remarks (derivatization,sheath liquid)	LOD/ *LOQ*/ µg/L	Ref.
RPLC	UV 260 nm	LiChrosphere 100	A: 0.1 M sodium dihydrogen-phosphate + 0.06 M phosphoric acid, pH 2.6 B: ACN	IDMP	fluorenylmethyloxy-carbonyl chloride	3.46	[38]
	PB-MS[f]	LiChrospher 100 Diol	A: isopropanol B: n-hexane	ATMP EDTMP DTPMP	diazomethane	6.6-13 8.4-19	[64]
	ESI-MS	ZORBAX Eclipse Plus C18	A: H_2O + 0.1% formic acid B: ACN + 0.1% formic acid	ATMP EDTMP DTPMP	trimethylsilyl-diazomethane	0.0025-0.06 0.008-0.02	[65]
CE	IPD[g] 259 nm	bare fused silica (45-55 cm length, 50 µm inner diameter)	BGE: 5 mM adenosine monophosphate, 100 mM boric acid, pH 7.10	glyphosate ATMP EDTMP DTPMP HDTMP[b]		1346-2006	[53]
	ESI-MS	bare fused silica (56 cm length, 50 µm inner diameter)	BGE: 10 mM trichloroacetic acid, pH 2.2	ATMP EDTMP DTPMP	sheath liquid 60:40 (v/v) MeOH: H_2O containing 20 mM trichloroacetic acid, pH 3		[66]
	ESI-MS	bare fused silica (64.5 cm length, 50 µm inner diameter)	BGE: 10 mM adenosine triphosphate disodium salt, pH 2.2	DTPMP ATMP EDTMP	sheath liquid 60:40 (v/v) MeOH: H_2O containing 20 mM trichloroacetic acid, pH 2.2		[67]

[a]PAD: Pulsed amperometric detection, [b]HDTMP: Hexamethylenediamine tetra(methylene phosphonic acid), [c]IRMS: Isotope-ratio mass spectrometry, [d]EABMP: Ethylaminobis(methylenephosphonic) acid, [e]published without addition of formic acid, otherwise same separation conditions, [f]PB-MS: Particle beam - mass spectrometry, [g]IPD: Indirect photometric detection

B) with MS detection:

The first LC-MS method for the analysis of ATMP, EDTMP, and DTPMP was published by Klinger et al. using an RPLC-column for separation and a particle-beam interface to the MS after derivatization with diazomethane.[64] LODs presented in this study were clearly lower with 7-13 µg/L compared to the best LODs reported for methods with other than MS detection (8.5-59.62 µg/L),[50] but the transfer of polar APPs into organic solvents for the derivatization by methylation was challenging. Furthermore, Klinger et al. stated that the presence of inorganic salts strongly decreased the sensitivity for environmental samples, especially after preconcentration using evaporation.[64]

Wang et al. followed a similar strategy making the phosphonates compatible with RPLC separation, but used trimethylsilyldiazomethane for derivatization instead of the explosive diazomethane, achieving good LODs of 0.0025-0.06 µg/L after preconcentration and purification combining a strong cation exchanger and a weak anion exchanger.[65] Using ESI-MS, not only the LOD was improved by a factor of about 100 compared to the method published by Klinger et al.[64] but the method was also applicable to environmental samples. The reported LODs are great for the targeted analysis of APPs, but due to the chemical changes caused by methylation, non-target screening of samples is difficult. In addition, the derivatization process was rather time consuming and required the use of carcinogenic and environmental harmful chemicals such as diazomethane and methanol.

To omit derivatization, direct analysis by HILIC, IC or CE coupled to MS detection is an interesting alternative. Coupling IC to an MS instrument requires special technical adaptations to ensure eluent compatibility with MS detection. When coupling IC to an MS instrument, a chemical suppressor is required to decrease background noise and salt load, which otherwise leads to the fouling of the ion sources due to salt built-up when using ESI-MS.[68] Furthermore, the addition of organic modifiers to the eluents can strongly increase ionization efficiencies when using ESI-MS.[52] For CE-MS analysis, a sheath-liquid is commonly used and a BGE made of volatile components is required.[69] In our work, we use an ESI needle made of platinum and iridium [70] to avoid corrosion and migration of metal cations into the separation pathway.[71] To ensure a closed electrical circuit during CE-MS analysis, e.g. a sheath liquid system is needed, delivering counter ions and supporting the low flow rates compared to LC (0.12-0.35 µL/min vs. > 0.1 mL/min).[72] Two CE-MS methods were published by one working group, demonstrating not only the ability to separate different APPs by CE, but also the applicability of the method to samples such as toothpaste. Interestingly, both methods used non-volatile BGEs in CE-MS coupling,[66, 67] which surely limits their long-term use. Concentrations of 2,000 µg/L investigated in this study were far above environmentally relevant concentrations (mostly <100 µg/L,[22, 23]) but may be suitable for purity testing of technical products or consumer products as shown in the examples in Fig. 3A, or laboratory studies on sorption or transformation kinetics. We recently investigated, if the method published by Wimmer et al. for the analysis of glyphosate and AMPA sediment and soil extracts [70, 73] could also be applied for larger aminophosphonates and other transformation products such as IDMP. However, a strong sorption was observed when using bare fused silica capillaries with an acidic BGE for larger aminophosphonates (see Section 3.4.2). Elaboration of different BGE modifiers such as adenosine triphosphate [67] or coatings which inhibit the adsorption of these phosphonate analytes is necessary.

Kuhn et al. and Riedel et al. also used ESI-MS for detection, but coupled to separations with HILIC. Two different HILIC methods with different stationary phases (see Table 1) were applied to samples, but no LODs/LOQs were reported.[42, 61-63] When combined with a high resolution MS, the methods reported by Kuhn or Riedel et al. cited above, could also be used for the identification of unknown transformation products, similar to the method adapted by Armbruster et al. with IC-MS.[52] In comparison with the IC method, however, a major disadvantage of both HILIC methods is the high consumption of organic solvent in the eluent.

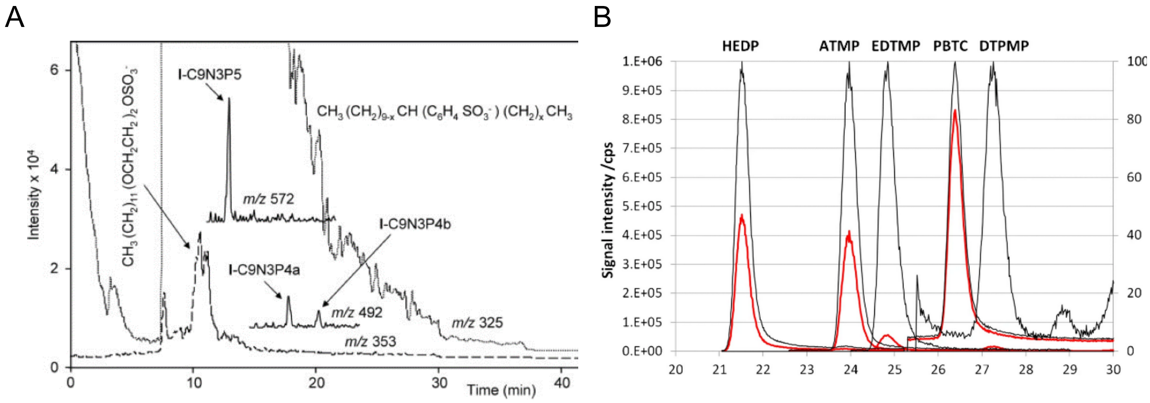


Fig. 3: (A) Electropherogram after CE-MS separation of a liquid cleaner for automatic cloth washers with a declared content of 0.1% of DTPMP in the technical product. m/z 572 is the mass of the nominal compound while other peaks are impurities or other product ingredients. Figure from Berbabé-Zafón et al., Electrophoresis, 28: 341-352, Copyright 2007, reproduced with permission from Wiley.[67] (B) Chromatogram of 10 µg/L phosphate multi-standard after IC-ESI-MS measurement including on-column-enrichment by Armbruster et al., Anal Bioanal Chem 412, 4807–4825 (2020), Copyright 2019, Reproduced with permission from Springer Nature.[52]

The lowest LODs of all analytical methods published so far were achieved by Schmidt et al. using IC-ICP-MS after preconcentration by solid phase extraction (SPE) with 0.002-0.025 µg/L. They also demonstrated the applicability of this method for surface and tap water samples without interference from matrix ions present in those samples.[57] Although this method is a great tool to quantify ATMP, EDTMP and DTPMP, it does not allow the identification of unknown transformation products, as its detection relies on the P content and information and no structural information can be obtained as in ESI-MS or NMR. For this reason, Armburster et al. adapted an IC method to be coupled with ESI-MS (exemplary chromatogram see Fig. 3B), achieving LODs of 0.04-0.16 µg/L, which were higher by a factor of 6-20 compared to ICP-MS detection.[52] The related loss in sensitivity compared to ICP-MS detection is compensated by the additional information gained by MS analysis, enabling not only the identification of target analytes by exact mass but also of unknown transformation products, even in environmental samples. Further applications of this IC-MS method are discussed in Section 2.1.3.3.

2.1.3.2 Sample preparation

As discussed in Section 2.1.3.1, an improvement of LODs and LOQs for APP analysis is not easily achieved. For enrichment, such as evaporation or treatment with ion exchange cartridges can help to improve LODs without changing the analytical method. However, the co-enrichment of matrix ions, especially other anions such as sulphate or phosphate,[57] may interfere with the analysis. During evaporation of aqueous samples such as drinking water, precipitation of phosphonate salts can be expected, which may be difficult to be redissolved.[62] In addition, inorganic salts present in wastewater, surface and drinking water were shown to strongly influence ionization during HILIC-MS analysis.[74] Thus, the removal of interfering ions during sample preparation is necessary when analyzing environmental samples,[61] but suitable protocols for sample preparation are still scarce. All methods listed in Table 1 used ion exchange for the preparation of environmental samples. Ideally, a separation of disturbing matrix components is possible by the analytical separation process. E.g. Wimmer et al. demonstrated the separation of disturbing anions such as chloride, sulphate, nitrate and phosphate from the analytes of interest glyphosate and AMPA (see Fig. 4) and the separation of other possibly interfering matrix components by using a background electrolyte of very low pH.[70] This gives rise to a high separation selectivity as carboxylic acids in organic molecules will mainly be uncharged. In a further study this method was applied to soil and sediment extracts and enabled the analysis of glyphosate and AMPA without using ion exchange cartridges. The sample preparation in this study was fast and easy with an extraction using a basic solution of 50 mM NaOH and Na_2HPO_3, where competition of phosphate with sorption sites enhanced the extraction of glyphosate and AMPA.[73] The extraction method was later applied to other transformation products of APPs: The use of high resolution MS enabled us to detect APP transformation products such as glyphosate, AMPA, *N*-methyl-AMPA and IDMP in sediment extracts (see Section 3.4.2 and Fig. 4). As the extraction method by Wimmer et al. is rather fast (ca. 2 h) [73] compared to the extraction method published by Armbruster et al. (ca. 5 h) [52] it is used in our laboratory also for routine analysis. Ideally, the method is further enhanced to also cover larger APPs. Then, the occurrence and distribution of APPs and their transformation products could be studied using only one method.

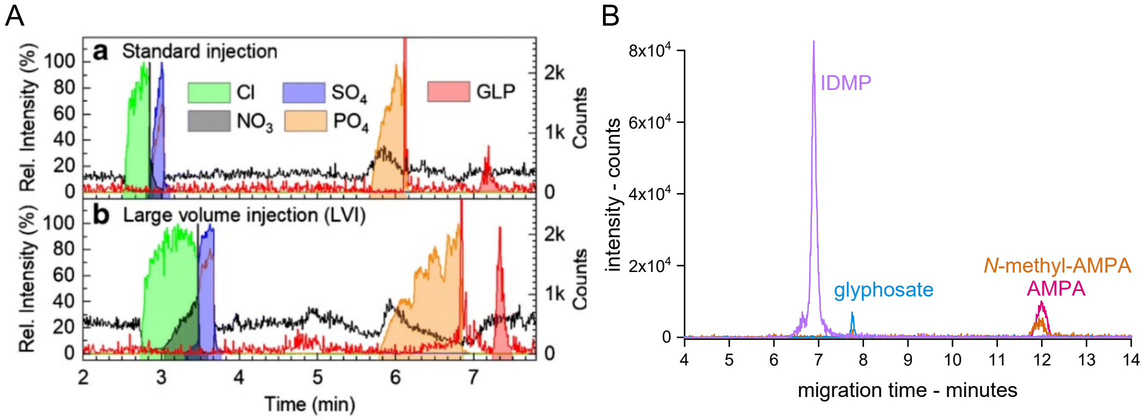


Fig. 4: (A) Electropherogram of degassed beer samples obtained using an acidic BGE using a capillary coated with polyvinyl alcohol to reduce analyte sorption. Source: Wimmer et al., Anal Bioanal Chem 412, 4967–4983 (2020), CC BY 4.0. (B) Electropherogram of a sediment extract from a sampling site in the receiving river Gründlach directly at the discharge of the WWTP Heroldsberg (detailed description of WWTP Heroldsberg in Section 5.3.2, extraction method in Section 5.3.3). Separation conditions and CE and MS instrumentation as described in Engelbart et al. were used.[44]

2.1.3.3 Applications to environmental samples

Most of the methods mentioned in Section 2.1.3.1 were used for laboratory experiments with ultra-pure water [41, 46, 50, 54, 64] or for the characterization of technical products.[33, 66, 67] In this context, concentrations are rather high. Commonly, only a few impurities were included in the analysis, e.g. minor substituted APPs.[33]

Jaworska et al. calculated an estimation of APP concentrations between 0.04-1.38 µg/L in surface water and 0.003-0.300 mg/kg in sediment for the Netherlands based on sales data and elimination rates.[23] Concentrations reported in different studies are: In German rivers, total phosphonate concentrations ranged between <0.1-39 µg/L in the aqueous phase and 1.8-51.4 mg/kg in sediments, with phosphonates being detected in sediment cores down to a depth of 66 cm in the Rhine by IC-MS.[22] An earlier study, Nowack used IC-UV after complexation with Fe(III) to detect ATMP, EDTMP and DTPMP in WWTP influent at concentrations between 21-974 µg/L and <LOD in effluent, only detecting DTPMP in one WWTP effluent at 80.22 µg/L, achieving LODs of 15-57 µg/L in drinking

water and WWTP influent and effluent.[75] Clearly, to gain a better understanding of the fate and occurrence of APPs in the environment, it is necessary to determine concentrations clearly below 1 µg/L and sediment concentrations below 100 µg/kg. The IC-ICP-MS method developed by Schmidt et al. separated APPs in algae medium and groundwater. With the adaptation of their method to ESI-MS detection, Armburster et al. were able to detect the analytes in extracts of sediments, solid matter from aeration tanks, river water, surface water, and also WWTP influent and effluent.[52, 57] With LODs and LOQs of 0.0025-0.06 µg/L and 0.008-0.02 µg/L, respectively, the RPLC-MS method by Wang et al. was capable to analyze APPs in tap water, surface water, WWTP influent and effluent.[65]

The HILIC methods developed by Kuhn or Riedel et al. were applied to the analysis of tap water, bacteria culture medium, supernatant of activated sludge, nanofiltration permeate and industrial laundry sewage water,[61, 62, 76, 77] but no LODs were stated.

2.1.4 Conclusion and Outlook

During the last 40 years, four basic strategies were followed for the separation of APPs: IC with or without ion pair reagents such as Fe(III), RPLC after derivatization, CE and HILIC. The direct methods are of interest due to the faster sample preparation without derivatization. Depending on the aim of the analysis, methods using detection techniques other than MS may be useful, e.g. for purity analysis of technical products, such as the most recent method published by Röhnelt et al.[50] However, these methods are inadequate for screening methods such as byproducts and impurities in technical products, where IC-MS was shown to be successful [52] and for the analysis of environmental samples, due to the relevance of transformation products and lower limits of detection required in combination with a high matrix tolerance.

Currently, the most promising method was published by Armbruster et al.[52] using IC-ESI-MS. Its applicability to various environmental sample types was demonstrated in studies by Rott et al.[22, 78] HILIC methods by Kuhn or Riedel et al.[42, 61-63] were not fully characterized with regard to LODs and not yet applied to extracts of solid samples such as sediments, which are, however, of great interest due to the strong sorption reported for APPs.[16] The advantage over the IC-MS method would be a better availability of suitable analytical equipment in many laboratories. Clearly, more advanced analytical methods have to be developed to further improve LODs and matrix tolerance, especially in complex matrices such as sediment extracts and wastewater. Currently, there is no method that covers both small and large phosphonates so the simultaneous analysis of parent and transformation products is not possible in environmental samples. Sample preparation schemes should be enhanced reducing costs and intense laboratory work, with a focus on reaching compatibility with direct analytical methods to those using derivatization. It has already been shown that CE-MS can be used after just extraction of the analytes and precipitation of humic acids and some metals as sole sample preparation steps prior to the analysis of transformation products with up to two phosphonate groups including IDMP and glyphosate.[70] Extraction methods for large phosphonates are not yet well elaborated as extraction efficiencies are unknown. More intense work would thus clearly require the availability of suitable isotope labeled internal standards and a better understanding of sorption effects.

Considering the analytical difficulties in the analysis of APPs in environmental samples, literature on their occurrence is scarce [22, 23, 75, 79] and neither the transformation processes nor their pathways in the environment are understood. Rott et al. clearly demonstrated the relevance for the investigation of APPs in the environment by detecting them at concentrations of up to 39 µg/L in aqueous phase and 51.4 mg/kg in surface water and sediments, which are high compared to other chemicals released from households and industry due to the high persistence of the phosphonates.[22] Even higher concentrations were observed, reaching up to 89 mg/kg extractable DTPMP directly at the discharge point of a WWTP (see Section 5.4.1). In this study, for the first time, also several transformation products were identified in sediment extracts, such as IDMP and *N*-methyl-AMPA, but currently our knowledge of the relevance of transformation products in the environment is insufficient. The only exceptions are glyphosate and AMPA, whose in-situ formation from DTPMP in activated sludge was demonstrated recently,[44] highlighting the importance to better understand APP transformation as even in this case, some knowledge gaps are still present regarding the importance of the agricultural vs. wastewater sources as discussed by Schwientek et al., whose study demonstrated the dominant input of glyphosate into surface waters via wastewater [5] and the in-situ formation of glyphosate and AMPA from DTPMP in activated sludge.[44]

2.2 Glyphosate contamination in European rivers not from herbicide application?

2.2.1 Abstract

The most widely used herbicide glyphosate contaminates surface waters around the globe. Both agriculture and urban applications are discussed as sources for glyphosate. To better delineate these sources, we investigated long-term time series of concentrations of glyphosate and its main transformation product aminomethylphosphonic acid (AMPA) in a large meta-analysis of about 100 sites in the USA and Europe. The U.S. data reveal pulses of glyphosate and AMPA when the discharge of the river is high, likely indicating mobilization by rain after herbicide application. In contrast, European concentration patterns of glyphosate and AMPA show a typical cyclic-seasonal component in their concentration patterns, correlating with patterns of wastewater markers such as pharmaceuticals, which is consistent with the frequent detection of these compounds in wastewater treatment plants. Our large meta-analysis clearly shows that for more than a decade, municipal wastewater was a very important source of glyphosate. In addition, European river water data show rather high and constant base mass fluxes of glyphosate all over the year, not expected from herbicide application. From our meta-analysis, we define criteria for a source of glyphosate, which was hidden so far. AMPA is known to be a transformation product not only of glyphosate but also of aminopolyphosphonates used as antiscalants in many applications. As they are used in laundry detergents in Europe but not in the USA, we hypothesize that glyphosate may also be a transformation product of aminopolyphosphonates.

2.2.2 Introduction

Glyphosate sales are expected to reach 900,000 metric tons worldwide in 2025.[80] In the USA, almost 130,000 t were used in 2012 in the agricultural sector [81] with 5-10% of the annual sales applied to non-agricultural sites.[81-83] Glyphosate and its main transformation product aminomethylphosphonic acid (AMPA) are frequently detected in rivers as well as in wastewater and sewage sludge.[40, 84-88] Glyphosate is commonly perceived to enter rivers via quickflow induced by rain events with loss rates after agricultural [11, 89] or urban applications [90-92] mostly reported to be below 1%. While the importance of urban sources has been discussed,[6, 11, 12, 93-95] we do not understand the significance of the various sources nor the input pathways of glyphosate and AMPA making it impossible to judge the effectiveness of recent mitigation measures in Europe.[96] To delineate sources of glyphosate and AMPA in surface waters, we examined long-term time series of river water contaminations. As already the first European datasets were in stark contrast to our expectations and common hypotheses of glyphosate entering surface waters via quickflow, we extended our study to conduct a large meta-analysis of river water concentrations across Europe and the USA. We compared concentration patterns with land use and correlated glyphosate and AMPA concentration patterns to those of other agrochemicals or micropollutants derived from wastewater.

2.2.3 Methods

Temporal patterns of glyphosate and AMPA concentrations in rivers and streams in Europe (E) and the USA (U) are compiled in Fig. 5, Fig. 6 and Fig. 7 and Table S1-1 and Table S1-2, which also provide information on the catchments (size, land use, impact by wastewater). The supplementary material provides additional figures and background information.

U.S. data: Sampling sites from the United States Geological Survey (https://maps.waterdata.usgs.gov/mapper/index.html) were selected based on the availability of long-term time series of glyphosate con- centration data with sufficient temporal resolution (≥12 samples per year), coverage of several states and contrasting land use (urban, agricultural, mixed), see Table S1-1. Data for pharmaceuticals or household chemicals were not available. Glyphosate and AMPA concentration patterns in Table S1-1 were plotted mostly with the same scaling, often using 1.5 µg/L as the upper value.

European data: Table S1-2 shows data plotted for 73 sites in France (38 sites), Sweden (3 sites), Germany (18 sites), the Netherlands (7 sites), the United Kingdom (1 site), Italy (2 sites) and Luxemburg (4 sites). From all available data, sites were chosen for which long time series with sufficient temporal resolution were available. We tried to cover sites all over the countries. Some sites were selected as they provide information on special aspects such as sites being impacted by wastewater treatment plants (WWTPs) receiving domestic wastewater. For European data, concentration time series were scaled according to the concentrations present at place. A comparison is made with other agricultural markers (mainly herbicides or nitrate) and wastewater markers (pharmaceuticals, especially carbamazepine, and household chemicals such as benzotriazoles or ethylenediaminetetraacetic acid (EDTA)).

The choice of the micropollutants was governed by the availability of data with regard to the type of micropollutant and sufficient temporal resolution for measured concentrations above the limit of detection. For agricultural markers, a focus was set to herbicides. Data handling: When plotting data, we decided to connect the data points (except when measured concentrations in the series were <LOD) to increase clarity of the plots. Most of the data are expected to be from grab sampling; in Table S1-2, we indicated the rare cases, where samples mixed over several days were used. In most cases, no detailed information on the sampling was provided with the data. We use the term “sharp concentration peaks” to indicate data points with concentrations clearly exceeding both, the preceding and the subsequent data point. In contrast, the term “broad concentration maxima” is used for wastewater-derived micropollutants and more persistent transformation products of herbicides like AMPA and dechlorometolachlor, which often show elevated concentrations over several sampling dates.

We applied Spearman rank correlation to relate glyphosate concentration data to concentration patterns of AMPA, wastewater and agricultural markers for selected sites.

The logarithm of the A:G ratio, log(A:G) proved to be an elegant measure to demonstrate the differences in the AMPA vs. glyphosate concentration patterns between the USA and Europe. This ratio indicates if there is a variable or more constant concentration ratio and which compound dominates over time.

2.2.4 Results

2.2.4.1 Concentration patterns in the USA

The general assumption is that glyphosate and AMPA enter rivers after herbicide application in conjunction with rain events.[97] All temporal concentration patterns and mass fluxes in the USA followed this hypothesis.

2.2.4.1.1 Catchments with a dominant agricultural impact

Several of the U.S. sites investigated here, have a dominantly agricultural catchment in sparsely populated areas with only small WWTPs, if any: site U7 (no WWTP), site U8 (impacted by irrigation), site U9 (small WWTP or private sewers, if any), site U13 (small WWTP <1,500 inhabitants in Hookerton), site U17 (no WWTP) and site U18 (small WWTP from a village with 2,300 inhabitants). Sharp concentration peaks, particularly for glyphosate are observed, exemplarily shown here for the South Fork Iowa River (Fig. 5A, site U7, other sites in Table S1-1). In many cases, glyphosate and AMPA peaks coincide with those of other herbicides such as metolachlor and are related with elevated discharge of the river. This clearly indicates rain-driven input as expected from agricultural runoff, likely due to first flush events after application.[89] AMPA patterns are more diverse with some sites showing predominantly sharp concentration peaks (e.g. sites U3, U5, U7, U15, U17) while others reveal broad concentration maxima over large parts of the growing season (e.g. sites U1, U4, U6, U9, U11, U16), see Table S1-1. Site U6, Bogue Phalia and U10, Yazoo River are described to have an intense use of glyphosate in their catchments,[98] which may lead to the accumulation of the more persistent AMPA.[39] This argument is supported when looking at the broad and similar concentration maxima of dechlorometolachlor, which is also more persistent than its parent metolachlor (Table S1-1).[99]

At the Sope Creek (Site U12) and at the South Fork Iowa River near New Providence (U7), the logarithm of the AMPA to glyphosate con- centration ratio, log(A:G), fluctuates around zero (median = 0.3) with either AMPA or glyphosate dominating at a time as can be expected for a small catchment, see Fig. S1. All sites have in common that winter times show lower concentrations and lower detection frequencies, especially for glyphosate. In all cases, similar input patterns are present for glyphosate and other herbicides. In Table S1-3, Spearman rank correlation coefficients between glyphosate and herbicides are often >0.6 (see also Fig. 8) (only for atrazine, lower values were often observed). Agriculture as a main source for glyphosate and AMPA can also be deduced when calculating mass fluxes, which increase during times of elevated discharge for glyphosate, AMPA and other herbicides (Fig. 6B and Fig. S3B). Agriculture as the dominant source for glyphosate input to surface waters was also discussed for Canada [100, 101] and Argentina.[102]

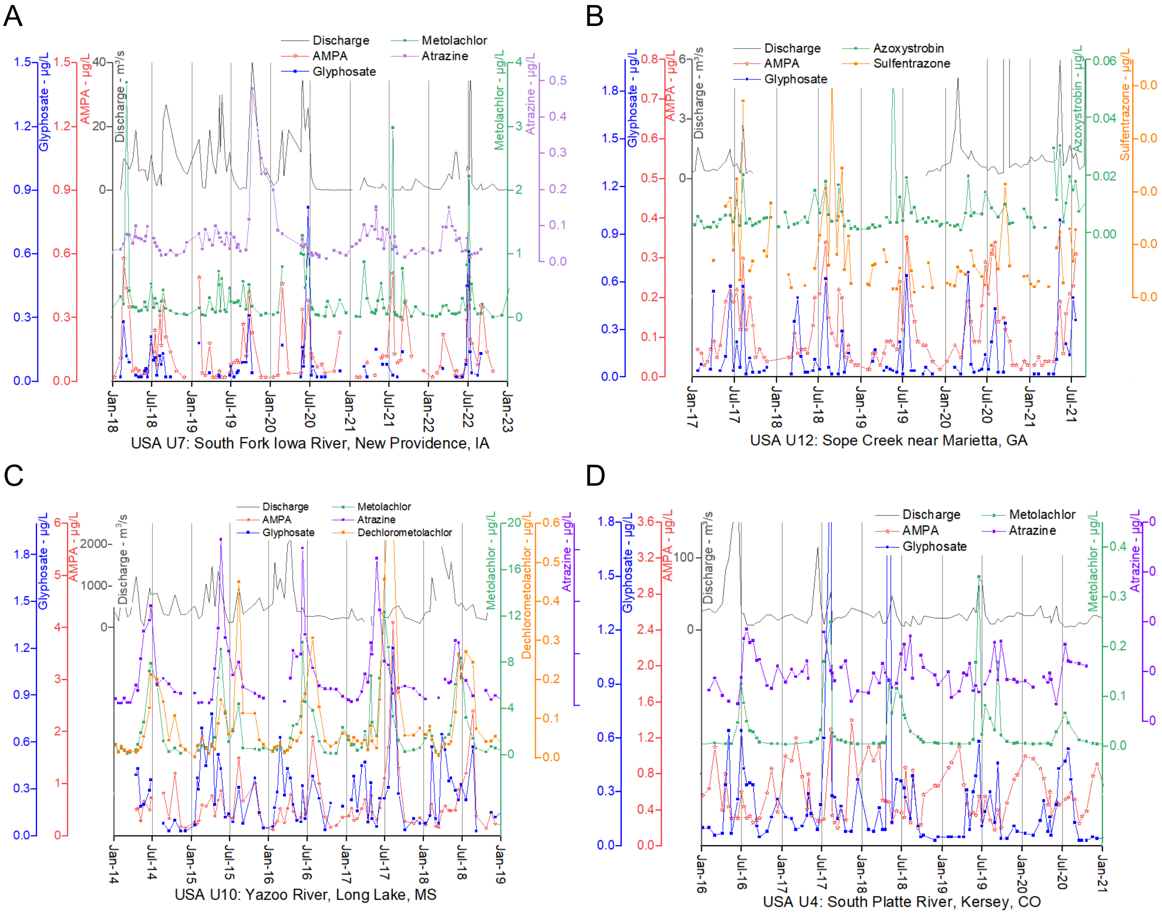


Fig. 5: Representative U.S. sites: Concentration patterns of glyphosate and AMPA and other herbicides as well as discharge in selected U.S. rivers. Details, data sources and additional data for 14 further sites are given in Table S1-1 (sites: U7, U12, U10, U4).

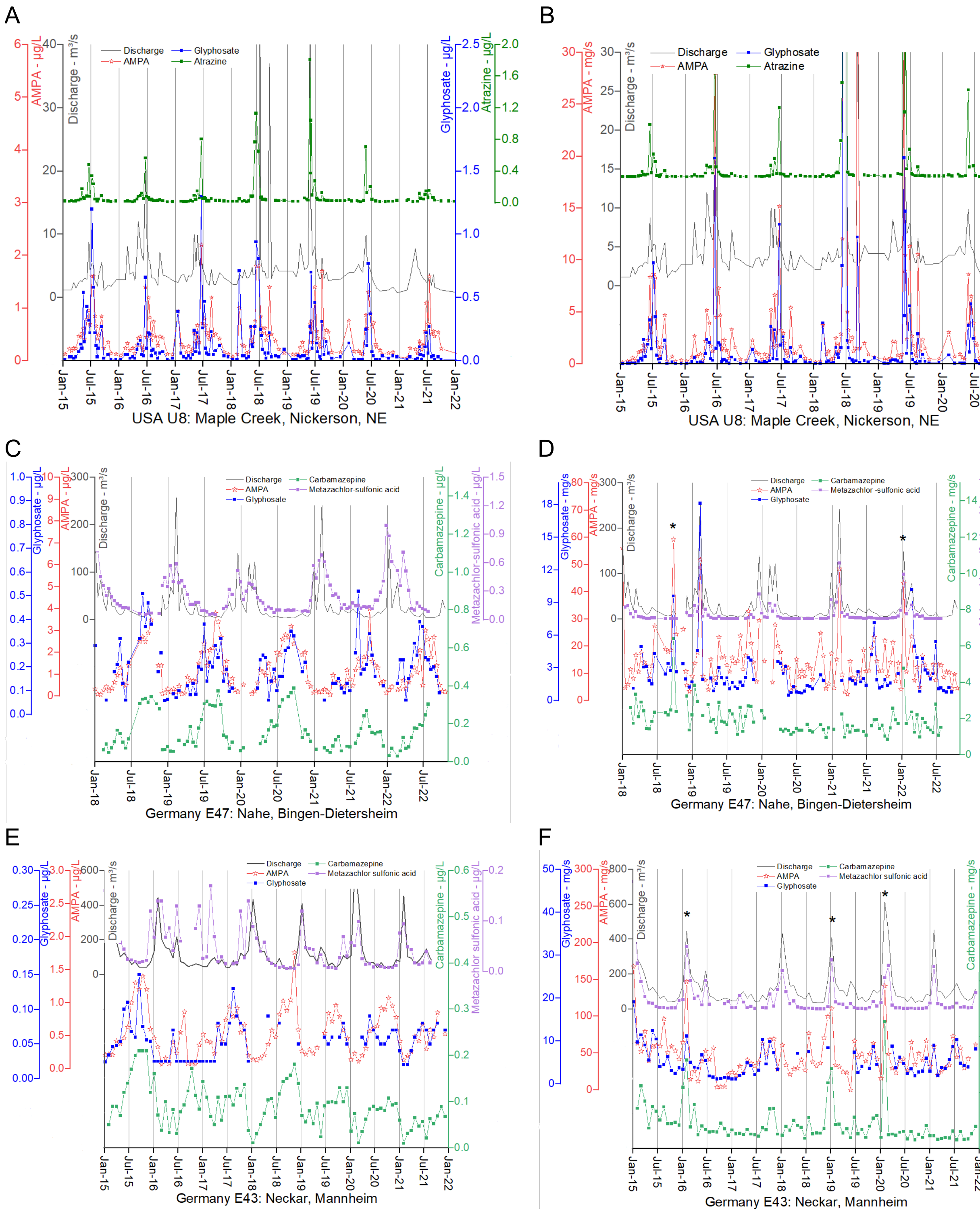


*Fig. 6: Concentrations vs. mass fluxes: A, C, E: Concentration patterns and B, D, F: mass fluxes of glyphosate and AMPA in the rivers A, B Maple Creek at Nickerson, NE (Site U8); C, D Nahe at Bingen (Site E47) and E, F Neckar at Mannheim (Site E44); data sources given in Table S1-1 and Table S1-2. *simultaneous increases in agricultural and urban tracers. Further examples in Fig. S3.*

Overall, we see strong differences between concentration patterns at different sites. Differences in the types of crop cultivated, management practices, catchment size and transport regimes for pesticides were discussed to be relevant for glyphosate input. As an example, we consider the work of Coupe et al., who provided application data and information on transport regimes for sites similar to some used in this meta-analysis.[98] For the South Fork Iowa River (close to site U7) and similarly for the White River basin (with the sites U17 (Sugar Creek) and U18 (White River) located in the same catchment), Coupe et al. described a dominance of subsurface flow due to artificial drainage in 80% of the catchment.[98] Here, glyphosate and AMPA detections were related to their main application times and to rain events. In contrast, at the Bogue Phalia (site U9), glyphosate and AMPA were detected during the whole growing season. This can be understood from the intense use of glyphosate use in glyphosate-resistant crop grown here, which allows applications over nine months of the year. Little drainage and a surface-water-driven system is present here and thus clearly different temporal input patterns.[98]

2.2.4.1.2 Urban catchments not impacted by wastewater

Similar concentration patterns with pronounced glyphosate peaks at elevated discharge are also present for rivers with fully urban catchments without wastewater impact (e.g., the Sope Creek in Marietta; Fig. 5B, site U12 and at Fanno Creek, site U1 (Table S1-1)), demonstrating intensive private and municipal use during the growing season (non-agricultural use is estimated to 5-10% of all sales [81-83]). For these sites, we also see a similar appearance of urban pesticides (e.g. Spearman rank correlation coefficients for glyphosate at the Sope Creek (U12) to azoxystrobin 0.606 and sulfentrazone 0.422, see Table S1-3) pointing to surface runoff as major input pathway, especially for site U12 with a significant per- centage of sealed surfaces (streets, driveways) in the residential area of the catchment.

2.2.4.1.3 Catchments with mixed land used and impact by wastewater

Also, for U.S. sampling sites with larger catchments and a mixed urban and agricultural input, most of them impacted by wastewater (U3–6, U10, U11, U14–16, details on WWTPs and disinfection protocols are provided in Table S1-1), similar concentration patterns are present. We included disinfection processes commonly implemented in U.S. WWTPs because chlorination was shown to efficiently eliminate glyphosate (and partially AMPA).[103-105] Many WWTPs were equipped with this technique in the USA, but its use declined from 95% in 1997 to 75% of U.S. WWTPs in 2003.[106] The alternative UV disinfection (21% of U.S. plants in 2003 [106] can be expected to be less efficient in glyphosate removal.[107, 108] Comparing data from different sites, we neither observe relevant differences in concentration patterns due to the type of disinfection nor differences in time upon changes in disinfection protocols, e.g. from chlorination to reaction with peracetic acid in Denver [109] (site U4, see Table S1-1). The sharp concentration peaks visible for sites U3, U5, U10, U14, U15 and the (continued) frequent switching of the log(A:G) from positive to negative values at many sites, see Fig. S1, demonstrate that the input of WWTPs does not principally change the concentration patterns in receiving waters. As glyphosate is only rarely detected and if, only at low concentrations in WWTP effluents in the USA,[10, 12] either efficient elimination may be present or input via the sewer system is of minor importance. In contrast, AMPA is more frequently detected in WWTP effluents. AMPA was consistently discussed to be a transformation product of aminopolyphosphonates (APPs),[26, 110] But this source would be expected to lead to rather constant base mass fluxes and an inverse relationship to discharge due to dilution, but the opposite is observed along with patterns consistent to other herbicides. Impressive examples can be found at Site U4 at Kersey (catchment 28,800 km^2, WWTP 2.2 Mio inhabitant equivalent (IE)) with up to 85% treated municipal wastewater in the South Platte River (Spearman rank correlation of glyphosate and metolachlor of 0.632, see Table S1-3) or at Site U6 at Hastings on the Mississippi (catchment 95,083 km^2, 1.8 Mio IE). At U6, concentrations patterns of glyphosate and metolachlor are similar with a Spearman rank correlation coefficient of 0.607 (n = 125). This also holds true for the patterns of AMPA and dechlorometolachor (0.648, n = 125).

2.2.4.2 Concentration patterns in Europe

By contrast, the features described for the USA are not at all representative of the European data (Fig. 7 and Table S1-2). The typical agricultural input patterns visible in the USA are rarely observed among the more than 70 sites investigated in Europe (e.g., at sites E2, E5, E24 (France), sites E39 (Fig. 7A) and E40 (Sweden), sites E61 and E65 (the Netherlands), see Table S1-2). For these sites, input patterns for glyphosate (reaching concentrations of up to 57 µg/L (Site E40 with a very small purely agricultural catchment)) and other agricultural markers (diflufenican (sites E2 and 5) or MCPA (E61)) resemble the hydrograph. In the large dataset available from France, we would expect agricultural concentration patterns especially in the sparsely populated headwater regions of river catchments, but detection frequencies and/or temporal resolution are too low.

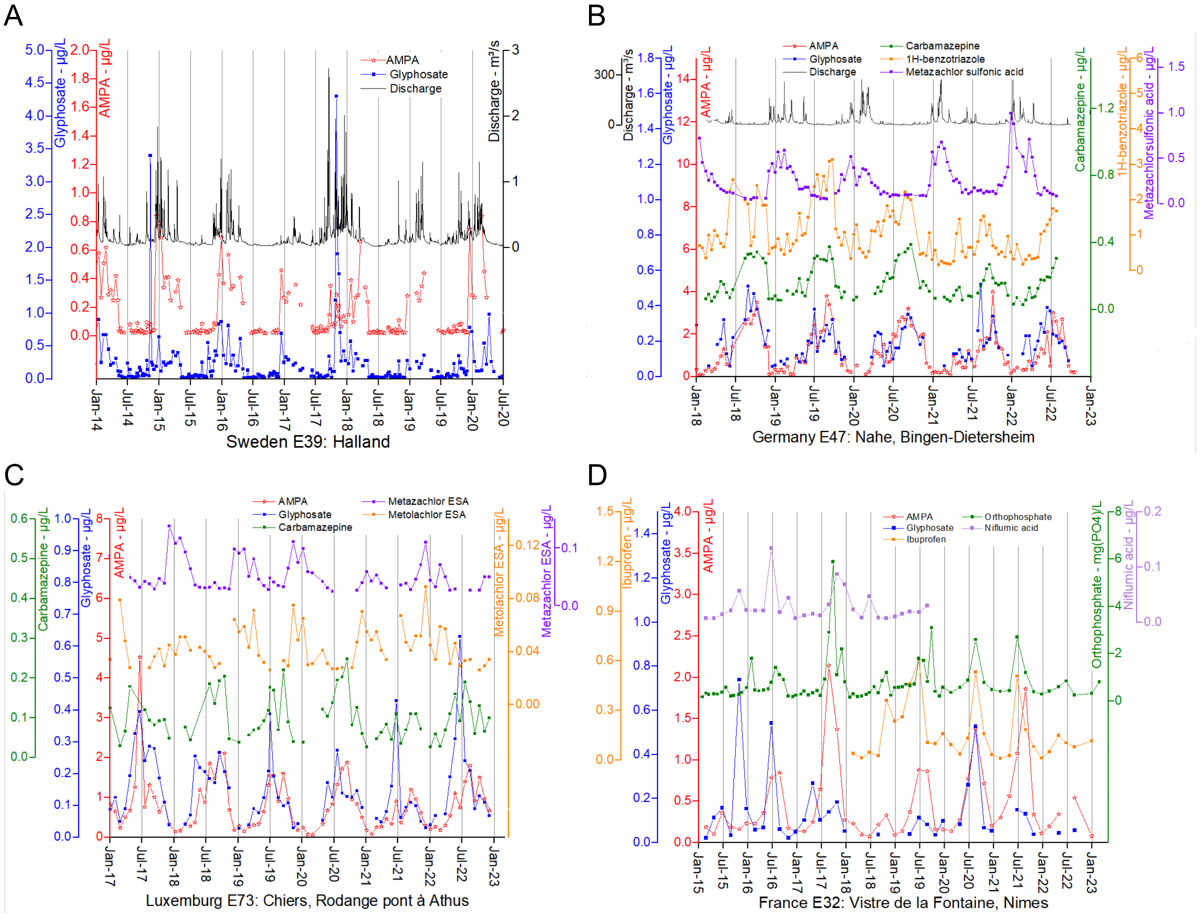


Fig. 7: Representative European sites: A-D: Concentration patterns of glyphosate and AMPA compared to concentrations of agrochemicals (herbicides, nitrate) or wastewater-derived substances (triazoles, pharmaceuticals, phosphate) and discharge where available in Swedish, French, Luxembourgish and German rivers. Details, data sources and additional data for almost 70 further sites are given in Table S1-2. Sites: A: E39 (SE), B: E47 (DE), D: E73 (LU), D: E32 (FR).

In contrast, most of the sites investigated, especially those with average concentrations >>LOD, show distinctly different patterns with a strong seasonality. Representative examples are shown in Fig. 7b-d (all other sites in Table S1-2). During winter months (November-March) with expected low use of glyphosate (see U.S. data), concentrations are lowest but often still well above LOD and with high detection frequencies. Concentrations regularly increase in April or May, reach a maximum mostly during July-October, when the discharge is lowest and then decline again (see Fig. 7 and Table S1-

2). The anticyclical patterns of discharge on the one hand and glyphosate and AMPA concentrations on the other hand is particularly well visible in Fig. 7b (Site E47, Nahe at Bingen-Dietersheim). Similar temporal concentration patterns were shown for sites in France,[111, 112] the Netherlands,[40, 94] and Switzerland.[6, 113] Seemingly, this similar contamination pattern all over (Western) Europe is independent from differences in land use (urban or agricultural), crop type, management practices or climate conditions, which surely prevail at the different sites (for catchment information, see Table S1-2). For example, site E29 (Aude a la Redorte, FR) has a catchment dominated by vineyards whereas the catchment for site E46 (Emscher, DE) is dominantly urban. Sometimes, sharp glyphosate peaks superimpose the seasonal pattern, but are limited to single events (sites E3, E10, E17, E18, E43 and E59). Glyphosate peaks are observed at sampling sites along the Helme (E53 a-d) at the same days, but in contrast to other points in time, AMPA concentrations did not increase in parallel, making rain-driven glyphosate input from the large neighboring fields likely.

For more than a decade (longest data sets reach back to 1997, Site E49), at most European sites, concentration patterns are not consistent with the main glyphosate application times for stubble and pre-sowing treatments in spring and late summer/autumn (for details, see Section S2). Genetically modified glyphosate-resistant crops are not approved in the EU. This limits summer applications of glyphosate to special crops (e. g. wine, legumes) or to pre-harvest (siccation) applications in crops such as oilseed rape, maize or cereals. Siccation applications, however, were strongly restricted since 2016 in Germany, fully banned there in 2021 and are now banned in the whole EU, see Section S4.[114] For Germany, it was stated that glyphosate was used on about 37% of agricultural land, but only on 2% for siccation (6% of all sales) in 2017.[3] Restrictions were implemented for municipal and private use (starting in 2017 in the EU) up to the full ban of glyphosate in Luxembourg from January 2021 until the ban was stopped again by a court decision end of March 2023. However, no reduction of glyphosate and AMPA contaminations in rivers can be seen (see Fig. 7C and Table S1-2 (sites E70–73)).

2.2.5 Discussion

2.2.5.1 Comparison of U.S. and European concentration patterns in rivers

We here summarize surface water data ranging from 1997 to 2023, mostly with 10 and more samplings per year for about 100 sampling sites in total. Samples at monthly intervals cannot clearly be attributed to distinct phases of processes such as the beginning, the peak, or the recession of a runoff event. However, we are confident that the high number of data points support more general conclusions despite the haphazard nature of grab sampling. This is supported by the strong differences seen in European and U.S. data. In addition, some time series reveal strong jumps in the concentrations of micropollutants at times of strongly elevated river discharge. This shows that in the large data set, both, base mass fluxes and increased mass fluxes during heavy rain events were sampled. European data reveal an approximately inverse relationship of glyphosate and AMPA patterns to discharge or nitrate as a marker for diffuse input from agriculture (e.g. sites E16, E17, E44, E47). The concentration patterns of other herbicides such as metolachlor and metazachlor and their transformation products clearly differ (sites E7, E15, E17, E22, E23, E25, E44, E47, E62, E70–73), indicated also by low to negative Spearman rank correlation coefficients, see

Fig. 8 and Table S1-4. This is in stark contrast to the USA (see Fig. 6A and B, Fig. S3A and B) and the agricultural catchment in Sweden (Fig. 7A, Fig. S3c-d), where glyphosate and AMPA concentrations and mass fluxes increased upon elevated discharge just like other herbicides, and are corroborated by high Spearman rank coefficients (

Fig. 8 and Table S1-3).

Glyphosate use is higher in the USA than in European countries with application rates in terms of total agricultural area of 138 kg/km^2 in the USA,[115] and of 26 kg/km^2 on average for European countries (ranging from 17 kg/km^2 for Luxemburg/UK to 32 kg/km^2 for France) [116] (details in Section S1-1). However, the concentration ranges of glyphosate in rivers are similar among USA, France and Germany (Fig. S2). European sites with a pure agricultural catchment have log(A:G) values fluctuating around a median of -0.1 to 0.1 over time similar to U.S. sites with small catchments (sites E39, U3 and U12, Fig. S1). By contrast, the log(A:G) ratios of most European sites are dominated by AMPA with values >1 (sites E3, E6, E8, E15, E16) and even >1.5 (AMPA concentrations >30 times glyphosate) for sites with larger catchments such as E33, E56, E62 (Fig. S1). Among the U.S. sites chosen here, median values up to 0.5 were only reached for the Red River (site U5, 70,000 km^2) and the Yazoo River (U10, 34,227 km^2). Here the fluctuations in log(A:G) were not as pronounced as in rivers with smaller catchments (see Fig. S1), likely due to the more frequent application of glyphosate in the catchment compared to small catchments. This finding is a first hint to a more constant source also for glyphosate present in Europe. Indeed, when calculating long-term glyphosate mass fluxes (Fig. 6C-F and Fig. S3E-F), we observe rather constant base mass fluxes for glyphosate and AMPA in Europe but not in the USA. This includes periods outside the growing season and even periods of extended droughts (e.g., summers of 2013 and 2018) when mobilization by rain is unlikely.

2.2.5.2 Glyphosate and AMPA entering surface waters via wastewater

A strong seasonality in concentration data and rather constant base mass fluxes are well known for micropollutants derived from wastewater such as phosphate, pharmaceuticals such as the antiepileptic carbamazepine or pain killers (niflumic acid or ibuprofen), and household chemicals (such as (benzo)triazoles used e.g. in dishwashing agents).[117] An impressive example is that of glyphosate and benzotriazole at the Teltowkanal (site E58, Table S1-2). Their seasonal concentration pattern can easily be explained by constant mass fluxes from a point source diluted during winter by river discharges elevated due to low evapotranspiration.[118] Unfortunately, suitable data for wastewater markers are lacking in the U.S. data. Comparative Spearman rank correlation analysis was performed for selected sites in the USA (7 sites, Table S1-3) and Europe (13 sites, Table S1-4). The distribution of the Spearman rank correlation coefficients is depicted as box-whisker-plots in Fig. 8. They demonstrate equally high correlations between glyphosate and AMPA concentrations for both continents, while herbicides were highly correlated with glyphosate only in the USA. In contrast, glyphosate concentrations at the European sites show a correlation with the wastewater-derived carbamazepine in a similar range as with AMPA but mostly low to negative coefficients for other pesticides, here mainly herbicides.

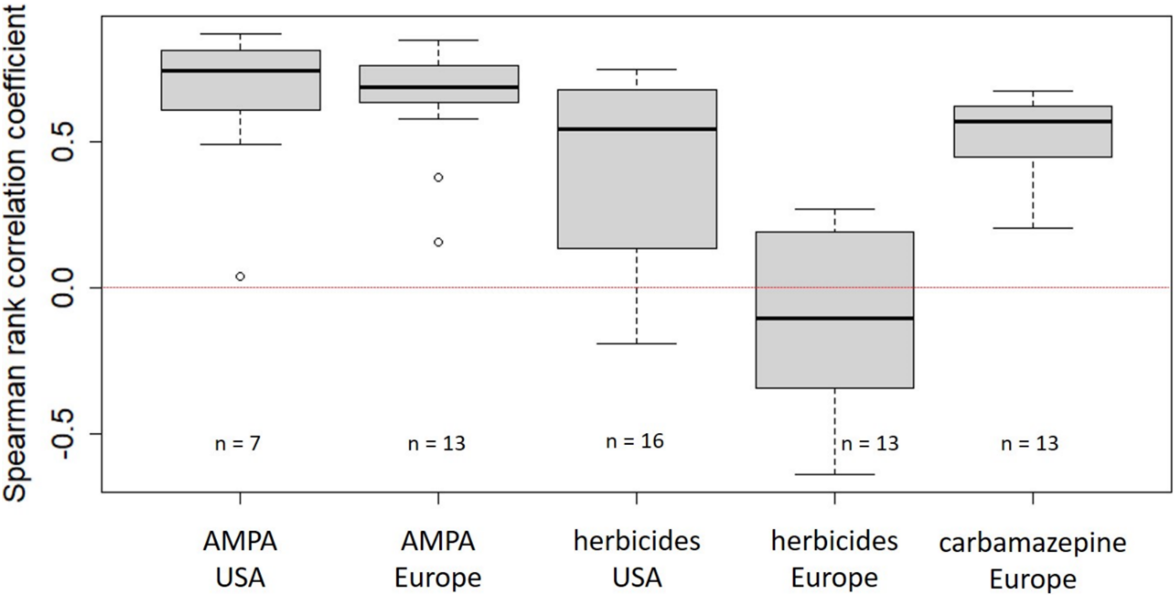


Fig. 8: Box-whisker-plots of Spearman rank correlation coefficients for rank correlation analysis of glyphosate with AMPA, with available data on herbicides and carbamazepine from selected sites in the USA and Europe. A correlation coefficient of 1 indicates a perfect positive, a coefficient of -1 a perfect negative relationship of the variables' ranks. The number of analyzed time series is indicated by n. Data in Table S1-3 and Table S1-4.

The relevance of wastewater for European river contamination by glyphosate and AMPA is further stressed by the fact that all European sites showing the seasonal concentration patterns are impacted by wastewater (see catchment information in Table S1-2). In addition, glyphosate concentrations increase upon passing a WWTP discharge point and decrease with distance to the next WWTP upstream (e.g. along the Seine (FR) (sampling sites Charrey-sur-Seine to Saint-Lye and Méry-sur-Seine and further downstream for Saint Fargeau-Ponthierry to Conflans-Sainte Honorine (site E18)) and at the Aude (sampling sites Trebes to La Redorte) (data not shown)). In Berlin, glyphosate and AMPA were hardly detected in the Dahme (site E57) but detection frequencies and concentrations strongly increased (to 0.05–0.5 µg/L glyphosate and 1–7 µg/L AMPA) in its branch Teltowkanal (site E59) after the discharge points of Berlin's largest WWTP Waßmannsdorf (1.3 Mio IE), WWTP Stahnsdorf (320,000 IE) and Ruhleben during summer months (1.6 Mio IE), see Table S1-2. The relevance of wastewater as a source is also visible by the number of positive detects in surface waters in Berlin (8%/ 35%/ 56% for glyphosate and 22%/ 55%/ 95% for AMPA) with no/ seasonal/ permanent wastewater inputs, respectively (Fig. 9) (wastewater discharge alternates into different rivers during the year).

For the USA, only very few data on glyphosate and AMPA concentrations in WWTP effluents were published: 1 of 11 (9 of 11) [84] and 3 of 11 (9 of 11) [12] effluent samples were tested positive for glyphosate (AMPA). The median glyphosate concentration was <LOD (LOD = 0.02 [84] and 0.1 µg/L [12]) and for AMPA, 0.45 µg/L [84] or <LOD [12] (LOD = 0.1 µg/L [12]) in the two studies. This is in strong contrast to Europe, where almost all WWTP effluents were tested positive for glyphosate and AMPA: In Switzerland, the median glyphosate concentration in 42 of 45 WWTPs was 0.34 µg/L with a range of 0.06–3.8 µg/L in 2016 (AMPA, 45 of 45 WWTPs, median 0.78 µg/L, range 0.054–8.40 µg/L). [88] Similarly, a German WWTP revealed a median glyphosate concentration of 0.55 µg/L (range <LOD to 5.4 µg/L) from monthly sampling (AMPA: median 1.35 µg/L, range 0.05–5.0 µg/L), data kindly provided by the Bayerisches Landesamt für Umwelt, Germany. WWTP effluents along the Meuse and its tributaries in the Netherlands had average concentrations of 1.6 µg/L glyphosate (up to 29.2 µg/L) (AMPA 3.5 µg/L, up to 50 µg/L) in 2010.[94] Poiger et al. detected glyphosate (and AMPA) from April to November in a Swiss

WWTP with average effluent concentrations of 0.16 µg/L (range 0.047–0.58 µg/L).[86] The most intriguing observations were made by Ghanem et al., who determined glyphosate and AMPA over one year in dried sewage sludge in a French WWTP with moderate industrial activity and fed by separate sewer systems: Concentrations reached up to 3 mg/kg glyphosate and 20 mg/kg AMPA,[87] see Fig. S4. Glyphosate and AMPA patterns were very similar to each other.[86, 87] A certain seasonality of concentrations in WWTP effluents, visible in the data of these two studies, may arise from sewer infiltration or storm water inputs during wet seasons or periods.[117] Hence, dilution effects may also occur in the sewer system but this does not contradict the assumption of rather constant mass fluxes. Finally, Märki et al. detected glyphosate in WWTP samples also during dry weather periods.[119] These findings question glyphosate contamination in streams to be derived only from rain-driven mobilization after herbicide applications. The rather constant log(A:G) ratios in receiving rivers, sometimes over decades, seem to reflect rather constant ratios in WWTP effluents.[86, 87, 119] Changes may be related to changes in the performance of WWTPs (e.g. at the Neckar in Mannheim (E44) and at the Main in Bischofsheim (E55).

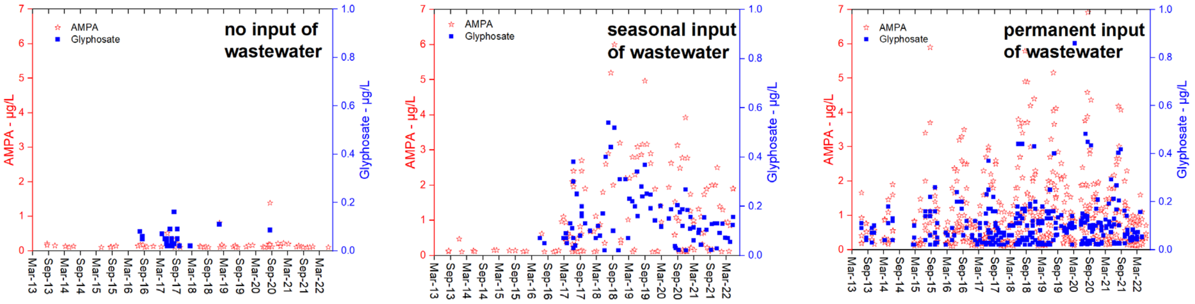


Fig. 9: Glyphosate and AMPA contamination in Berlin surface waters, plotting data for several rivers as point clouds classified regarding the temporal patterns of wastewater input. Data and information kindly provided by the Berliner Wasserbetriebe.

A study at the Meuse in 2010 suggested that wastewater is a dominant source of glyphosate contamination: loads in the Meuse at a sampling point close to the French border in Tailfer (650,000 inhabitants in the catchment) were 0.28 kg/day glyphosate and 1.28 kg/day AMPA.[94] Close to the estuary at Keizersveer (7.7 Mio inhabitants in the catchment), loads increased to 0.9 kg/day glyphosate and 19 kg/day AMPA. Thus, for glyphosate, the loads increased by 0.62 kg/day. A significant fraction of this increase in glyphosate mass flux can be explained by input via WWTPs: an additional load of 0.7 kg/day glyphosate was determined for several (25) but not all WWTPs discharging into the Meuse and its tributaries.[94] For AMPA, this load from the WWTPs was 1.36 kg/day. APPs were discussed to be an additional source, especially with regard to one tributary with very high AMPA loads (3.7 kg/day) presumed to stem from the use of phosphonates as antiscalants in cooling waters of chemical industries.

Our meta-analysis provides indications, that combined sewer over- flow may be a relevant source for peak concentrations of glyphosate and AMPA in rivers. We see events of elevated discharge, where glyphosate and AMPA concentrations increase together with both wastewater and agricultural markers (see asterisks in Fig. 6d and f). A sampling with a very high temporal resolution during heavy rainfall in France showed glyphosate and AMPA concentrations to increase simultaneously with those of fecal indicators due to sewer overflow but hardly with the subsequent concentration peak of agrochemicals.[120]

2.2.5.3 An unknown source for glyphosate?

The importance of urban sources for glyphosate and AMPA has been discussed before,[9, 94] especially in the Netherlands.[40, 94] As mentioned, AMPA is a known transformation product also of APPs, which are intensely used in Europe as antiscalants, bleach stabilizers, and corrosion inhibitors mainly in laundry products, in the textile and paper industries, and in cooling circles.[26, 34, 56, 110, 121] AMPA formation from APPs in WWTPs was discussed by Wang et al.[65] We may thus assume that APPs are the dominant source for AMPA. Then, the impressive differences between U.S. and European river contamination patterns and residues in WWTPs can easily be explained for AMPA: Opposite to Europe, the most popular U.S. laundry detergent brands do not contain APPs (web search 6/2023). Sales numbers for APPs were reported to be significantly lower in the USA compared to Europe.[18, 19]

But how to explain the findings for glyphosate? The common perception is that glyphosate enters WWTPs after private or municipal urban herbicide applications, or from applications along railway tracks. However, looking into more detail (see detailed discussion in Section S1-3), none of these applications would explain rather constant base mass fluxes all over the year, especially not during long dry periods. E.g. in Germany, the number of permits for municipal and industrial glyphosate applications are very low and comprise maximal two applications during the growing season (Section S1-3.1). Similarly, railway tracks were reported to be treated only once per year [122] with low findings of glyphosate at larger distance to the tracks.[123] Sorption to soil particles and thus lowered bioavailability for transformation as well as possible long sludge retention times in WWTPs could be expected to broaden peak input after applications and rain events, but this is clearly not observed in the USA despite intense urban and agricultural use. Urban use in the EU became more and more restricted in recent years but mitigation strategies did not change surface water concentrations (see Section S1-4). Input via diet and urine would be a possible constant source for glyphosate in WWTPs, however, modeled loads for this source are too low to explain field data (see Section S1-3.3).

Some rough model calculations may aid to judge the loads that can be expected from urban herbicide applications. We can assume 80–90% elimination rates in WWTPs [86] and low loss rates of 1-2% reported for glyphosate from residential areas [90, 91] (see also Section S1-3.1). At the Teltowkanal in Berlin (site E59), the average yearly load of glyphosate in the canal is 28 kg/year (2015–2021). Considering elimination and loss rates, we can estimate an amount of the herbicide theoretically applied in the range of 2.8–28 tons of glyphosate per year in the catchment. This is high with regard to sales numbers for non-occupational use in Germany having declined from 95 tons per year in 2014 to 17 tons in 2021 (statistics from the German Bundesamt für Verbraucherschutz und Lebensmittelsicherheit 2022). With the estimate, a theoretical area of 41-390 km^2 could have been treated in the catchment of the WWTPs Waßmannsdorf and Stahnsdorf (and seasonaly Ruhleben, see Section 2.2.5.2) (calculated using: recommended doses of 0.17 g/m^2 (garden applications) or 0.072 g/m^2 for agricultural use (application in volunteer grain)). For comparison, the total area of Berlin is about 1,000 km^2. Similarly, we estimate a load of 8 kg/year glyphosate at Site E54 with an old WWTP near a small village (500 inhabitants). Calculating with only 50% elimination for the two sewage ponds, 0.8–1.6 tons of glyphosate and a theoretical application area of 4.7–9.4 km^2 are estimated. The area covered by the village is only 0.7 km^2 (simply using a rectangle in the map). We want to stress that to explain surface water concentrations, the application of glyphosate must evoke a rather constant input throughout the year.

For comparison, the model calculation can be reversed: If we estimate urban glyphosate use from sales numbers for non-occupational use to 10–100 tons of glyphosate (a broad range to account for the high uncertainty) (Section S1-3), a loss rate of 1%, 80% elimination rate and 10 billion m^3 wastewater in Germany, we could expect average WWTP effluent concentrations of glyphosate of 0.002–0.02 µg/L. This is clearly lower than the concentrations observed in European WWTPs (Section 2.2.5.2) and often even lower than river water concentrations (Table S1-2 and Fig. S2), for which further dilution by mixing of the WWTP effluent with river water would have to be considered.

Our meta-analysis clearly shows that municipal wastewater is important (see discussion for the Teltowkanal, Site E58), but provides further hints that domestic wastewater must be relevant: At site E53a (catchment only ca. 25 km^2), the seasonal pattern of AMPA is clearly visible and slightly indicated also for glyphosate. The site is about 8 km downstream of the Helme spring and downstream of the small WWTP of Stöckey (400 inhabitants). There is no industrial input. Similarly, only wastewater from households is relevant for sites E16, E41 and site E19 (600 m downstream of the Aubance spring in the village of Louerre (500 inhabitants). Finally, clear seasonal patterns of glyphosate and AMPA (concentrations up to 0.8 and 2 µg/L, respectively), flanked by the patterns of painkillers and phosphate are visible at the Vistre de la Fontaine in Nîmes (site E32, catchment of 41 km^2) with its spring in the city center. The river is mainly diverted through still existing

Roman sewers used as the modern city's sewer system for a long time. It is known that some houses are still connected to this old sewer system,[124] making domestic wastewater a likely constant source for glyphosate.

2.2.6 Conclusion

Our meta-analysis on U.S. and European river water concentrations and additional information presented show that the dominant source for glyphosate in Europe cannot be herbicide application but is wastewater - the major indications being that, **1)** in contrast to the USA, seasonal patterns in Europe are not consistent with a dominant input from agricultural or urban herbicide applications. **2)** Only in Europe, rather constant base mass fluxes of glyphosate are present even during long dry summer periods and outside the application period of herbicides. **3)** Glyphosate and AMPA are detected in WWTPs connected to separate sewer systems receiving mainly domestic wastewater [87] and during dry weather periods.[119] **4)** High and constant loads shown to stem from WWTPs are difficult to relate to urban herbicide use. **5)** Model calculations for WWTP effluent concentrations of glyphosate from sales for non-occupational use are much lower than actual field data. **6)** Mitigation strategies did not change surface water concentrations or patterns. **7)** Concentration patterns of AMPA and glyphosate are very similar, which is unexpected given the different input pathways for AMPA, which are related to surface runoff (formation from glyphosate) and municipal wastewater (formation from APPs).

What might this as yet unknown source for glyphosate be? Our results give rise to the following criteria:

1) A discharge into watercourses via WWTPs;

2) An origin in municipal and domestic wastewater;

3) An application/usage over the entire year;

4) An application/usage in most (Western) European countries but not in the USA;

5) A source for both glyphosate and AMPA; and

6) Relevant since at least 1999 (see site E49, Selz at Ingelheim).

We are not aware of any technical or domestic glyphosate applications evoking a constant input into wastewater and rivers leading to a rather constant log(A:G). As discussed, all aspects of this meta-analysis regarding AMPA concentration patterns can well be explained by its formation from APPs. However, accepting APPs as the dominant source for AMPA in Europe, raises the hypothesis that also glyphosate originates from these chemicals, making APPs used e.g. in laundry detergents a common precursor for both AMPA and glyphosate. This hypothesis is further substantiated by the lack of APPs in U.S. detergents and by the work of Klinger et al.,[125] demonstrating the formation of glyphosate during ozonation of the APP EDTMP already in 1998. Our ongoing experimental work addresses the formation of glyphosate under environ- mentally relevant conditions.

3 New Approach on enrichment and analytical methods for aminopolyphosphonates and possible transformation products

3.1 Abstract

Glyphosate is known as the world's most widely used broad-spectrum herbicide. It is frequently detected in surface waters, especially in receiving rivers of wastewater treatment plants. Other aminophosphonates with high sales numbers are aminotris(methylenephosphonic acid) (ATMP) and diethylenetriaminepenta(methylene-phosphonic acid) (DTPMP). Recently, the formation of glyphosate from DTPMP was demonstrated together with a broad spectrum of other transformation products. Currently, there is no analytical strategy to analyze both small and large aminophosphonates with one method in environmental samples.

The current standard procedure for glyphosate analysis includes the time-consuming derivatization followed by LC-MS/MS detection, reaching LODs in the low ng/L range. Direct analysis of glyphosate by capillary electrophoresis-MS (CE-MS) minimizes sample preparation and analysis time, but LODs are not yet sufficient to monitor glyphosate in surface waters. Thus, glyphosate has to be enriched prior to CE-MS analysis. In this study, a new approach for the enrichment of glyphosate is tested by using soil minerals as sorbents combined with an intense optimization of the extraction medium to address different sorption sites on the mineral surface. The iron oxides goethite and magnetite, the clay mineral illite and the aluminum oxide Alumina-A were investigated regarding sorption isotherms and extractability of glyphosate. The most promising results were obtained by using magnetite as a sorbent and an extraction medium at elevated pH containing phosphate to compete for sorption sites, but also oxalic and ascorbic acid to etch the mineral surface while releasing glyphosate. An enrichment factor of 5 and a recovery of 64.9% were reached.

In order to be able to analyze large aminophosphonates together with their transformation products, a CE-MS method was adapted with the challenge to deal with the strong sorption especially of the polyphosphonates. In addition, hydrophilic interaction liquid chromatography (HILIC)-MS separation methods were investigated.

3.2 Introduction

Glyphosate and larger aminopolyphosphonates (APPs) are anthropogenic compounds widely used around the globe. Glyphosate is known as the world's most widely used broad-spectrum herbicide with annual sales of 4,000 tons/year in Germany in 2019-2022,[126] despite increasing restrictions set for its application. APPs such as aminotris(methylenephosphonic acid) (ATMP), ethylenediamine tetra(methylene phosphonic acid) (EDTMP) and diethylenetriamine penta(methylene phosphonic acid) (DTPMP) are used as chelating agents and antiscalants. In 2004, the reported European total quantities for phosphonate use ranged from 10,000-50,000 tons, of which 12,000 tons were used in household products (among them estimated 4,100 tons of DTPMP).[15] Their applications are divers, such as domestic and industrial laundry detergents and cleaning products,[25] where they function as bleach stabilizers and anti-scaling agents and corrosion inhibitors. Industrial applications include among others food industry, membrane treatment in reverse osmosis in drinking water production [26-29] and desalination,[30] and the prevention of scale formation and biofouling in cooling water systems.[31] In addition, they are commonly used as plasticizers in concrete [32] and in the paper, metal, and textile industries.[18] Rott et al. reported the occurrence of phosphonates in a wastewater treatment plant (WWTP) with dissolved phosphonate concentrations from 131-384 µg/L in the WWTP influent and 13-59 µg/L after the secondary clarifier and an adsorbed phosphonate load of 223-2,555 mg/kg in the influent.[78] The same group of authors detected phosphonates in the receiving rivers of WWTPs both dissolved and sediments. A significant effect of WWTP effluent on the phosphonate load of a receiving river previously not impacted by wastewater was demonstrated.[22] An earlier study of Nowack et al. also demonstrated the presence of phosphonates in wastewater influent and effluent of swiss WWTPs.[47, 75] Despite their widespread application and intense consumption worldwide, comparatively little is known about their environmental fate and relevance and especially about the formation, occurrence and relevance of their transformation products.

This is partly due to the fact that the analysis of such polar and highly charged compounds with their strong tendency to sorb to oxidic surfaces such as minerals or glassware, is still challenging. The analysis of glyphosate and AMPA is advanced with the DIN ISO 16308 (2017-09) [127] describing their analysis after derivatization with fluorenylmethyloxycarbonyl chloride (FMOC-Cl) and subsequent LC-MS analysis as the standard method. But only a few methods have been published for the analysis of polyphosphonates so that no standard method using MS detection has yet been established.

An example for a chromatographic separation of the polyphosphonates with MS detection are methods with hydrophilic interaction liquid chromatography (HILIC)-MS [61, 63] and ion chromatography (IC)-MS [33, 57], both derivatization-free. The analysis of aqueous samples from phototransformation experiments, industrial wastewater and sediment extracts was achieved. An alternative is to use derivatization to increase hydrophobicity and reduce charge to make the analytes compatible with reversed phase liquid chromatography (RPLC). For example, ion-pair HPLC after transformation of phosphonates to Fe(III)-complexes while using UV detection was implemented by Nowack et al.[47] Wang et al. methylated the phosphonate groups of 2-phosphonobutane 1,2,4-tricarboxylic acid (PBTC), etidronic acid, ATMP, EDTMP and DTPMP prior to separation using an RPLC column with derivatization agented by trimethylsilyldiazomethane.[65] The method was applied to quantify phosphonates in tap and river water, domestic and industrial WWTP influent and effluent.[65] However, the analysis of DTPMP using this method was impaired by matrix effects and therefore standard addition had to be used for quantification. Another disadvantage of this method is that it predominantly targets large, intact polyphosphonates. In our work, we showed that smaller phosphonates such as glyphosate and AMPA cannot be derivatized with sufficient yield (data not shown). Furthermore, the process of derivatization proved to be time consuming and needed a strict control of the sample pH. The presence of cations can inhibit complete derivatization.[65] The application of HILIC and IC methods requires a complex and expansive sample preparation mainly to due to the need reduce the ionic load when analyzing e.g. industrial laundry sewage,[61] sediment extracts or suspended matter of wastewater.[52] The sample preparation included the use of strongly acidic ion exchange cartridges such as Dowex 50WX8 [61] or ion exchange resins like AG MP-50 (H^+ form).[52]

The direct analysis of glyphosate and aminomethylphosphonic acid (AMPA) by capillary electrophoresis (CE)-MS was shown to minimize sample preparation and omitted derivatization.[70, 73] Analyzing these compounds in soil or sediments required only the phosphate-supported basic extraction, acidification to precipitate humic acids and metal cations and centrifugation. For beer samples, the LOD was 5 µg/L [70] and 9-15 µg/kg for soil extracts.[73] A high separation selectivity was reached with an acidic separation buffer so that e.g. many carboxylic acids are not charged and cannot impact the analysis. CE-MS was shown to be a useful separation method for the analysis of glyphosate and AMPA in baby food, beer, soil, sediment, and activated sludge from WWTPs.[44, 70, 73, 128]

Regarding the analysis of large polyphosphonates and smaller phosphonates, important to better understand the fate and transformation of these compounds, only a CE method with indirect photometric detection was published. It proved to be able to separate both the small aminophosphonates glyphosate and AMPA but also the large APPs ATMP, EDTMP, hexamethylene diaminetetra(methylenephosphonic acid) (HDTMP) and DTPMP.[53] Unfortunately, the method cannot be transferred to CE-MS due to the use of a non-volatile background electrolyte (BGE). A promising method analyzing polyphosphonates samples and impurities therein used CE separation with either indirect photometric detection or MS detection. For CE-MS, however, only high analyte concentrations of e.g. 2 g/L DTPMP were applicable,[67] possibly due to the use of adenosine triphosphate and diethylenetriamine as BGE components presumably to reduce sorption, which likely impair sensitivity. These high concentrations are not relevant in environmental samples. But the good separation selectivity achieved in these studies, makes CE an interesting method for the rapid and derivatization-free analysis of APPs and their transformation products.

The methods published so far strongly differ in the limits of detection reached: The lowest reported LOD reached for glyphosate analysis in extracts of baby food by CE-MS was 0.5 µg/L, using sheathless electrospray ionization.[128] This is clearly higher than the LODs reached with the current standard analytical method RPLC-MSMS, which are between 1-20 ng/L after FMOC-derivatization.[6, 8, 100, 129-133] The DIN ISO 16308 (2017-09) seeks a range of 0.03-1.5 µg/L for both glyphosate and AMPA in the dissolved faction of drinking water, ground water and surface waters (except marine or saline water) using RPLC-MSMS.[127] To meet these low LODs with CE-MS, enrichment of glyphosate from the aqueous phase is necessary. Enrichment methods published often chose solid phase extraction (SPE) via cartridges with Oasis HLB,[100, 134-138] Strata X [7, 129, 136] or molecular imprinted polymers.[132, 139] These commercial solutions strongly increase costs and generate a lot of waste. In this study, minerals commonly present in soil and sediments are investigated as a more sustainable sorbent for glyphosate enrichment. These materials may also be suitable for passive sampling of glyphosate in water as an alternative to the use of expansive molecular imprinted polymer membranes [140] or disks using polymer-based sorbents [141] with problems regarding long-term stability in soil media.

Strong adsorption of glyphosate on Al-(hydr)oxides, and Fe-(hydr)oxides and the phyllosilicate illite was reported.[142-144] Illite is a three-layer silicate (2:1) and consists of two tetrahedral layers with an octahedral layer in between. Besides the isomorphous Si^{4+}-Al^{3+} exchange in the tetrahedral layers, exchange of Al^{3+} with Mg^{2+} or Fe^{2+} in the octahedral layers is possible, resulting in a permanent negative charge. This is commonly balanced by depositing K^+ in the interlayers. This causes the layers to contract. Due to the dominance of silanol groups (Si-OH), the pH of zero charge (pH_{PZC}) for illite is 2-3.[145] Similarly, oxides such as Al_2O_3, goethite, and magnetite contain hydroxyl groups (M-OH, $M\text{-}O^-$) and their surface charge depends on the pH of the environment. The pH_{PZC} for oxides and hydroxides is approximately 8.[146] Sorption of anions, such as glyphosate or phosphate, is described to occur via ligand exchange, H-bonds, or complexation of metal ions on the surface. In the case of ligand exchange, the hydroxyl group is protonated and

H_2O is lost, resulting in the formation of a tightly bound inner sphere complex upon the formation of mono- or bidentate complexes.[145] It was shown that glyphosate addresses different sorption sites depending on the mineral composition.[142, 144, 147-149] For extraction of glyphosate after the sorption phase, these differences have to be addressed by a careful choice of the pH and composition of the extraction medium. For example, desorption is supported by coulomb repulsion between glyphosate and mineral surface at a very high pH.[150] Competition with phosphate-based compounds for binding sites relevant for the phosphonate moieties of glyphosate was shown to be important to reach high recoveries. [151] Finally, a partial dissolution of minerals via etching the surface using reductants such as oxalic or ascorbic acid was used.[152, 153]

Therefore, this study provides a new approach for the enrichment of glyphosate and AMPA using natural minerals common in soil and sediment as sorbents and different extraction media compatible with downstream CE-MS analysis. Second, new analytical approaches for the simultaneous separation and detection of DTPMP and EDTMP and smaller amino(poly)phosphonates from their transformation including glyphosate and AMPA, are investigated. This method could also be an alternative to already published HILIC methods,[41, 61] which are rather time and material consuming. The results of this study will enable us to better understand the occurrence and transformation of amino(poly)phosphonates in the environment.

3.3 Materials and Methods

3.3.1 Chemicals

All chemicals were used without further purification.

Ultra-pure water (≥18 MΩcm^{-1}) used throughout this study was obtained for LC-MS1 and CE-MS using an ELGA-Veolia PURELAB Classic system (Celle, Germany) or for LC-MS2 using an ELGA-Veolia PURELAB flex 1 system (Celle, Germany).

3.3.1.1 Analytical standards

DTPMP standard as free acid powder under the name “Cublen D 900 GR” with an active acid content of >90% (CAS: 15827-60-8) and EDTMP under the name “Cublen E 34135 P” with an active acid content of 34% (CAS: 85480-89-3) were purchased from Zschimmer & Schwarz Chemie (Lahnstein, Germany) and their purity was controlled by ^{1}H and ^{31}P-NMR. AMPA (≥99%), and iminodi (methylphosphonic acid) (IDMP) were purchased from Sigma-Aldrich (Steinheim, Germany). *N*-acetyl-(aminomethyl)phosphonic acid (*N*-acetyl-AMPA), *N*-methyl-(aminomethyl)phosphonic acid (*N*-methyl-AMPA) (≥99%), glyphosate and *N*-acetyl-glyphosate (*N*-acetyl-glyphosate) (95%) were obtained from TRC (Toronto, Canada). Glufosinate-ammonium was bought from LGC (Wesel, Germany). For quantification glyphosate-2-^{13}C (≥99.9%) (G169) and glyphosate-2-^{13}C-^{15}N (G170) (≥99%) from Sigma Aldrich (Steinheim, Germany) were used.

For method development a mixture of analytes (AM) was used consisting of glyphosate, AMPA, IDMP, *N*-acetyl-AMPA, *N*-acetyl-glyphosate, *N*-methyl-AMPA, glufosinate, DTPMP and EDTMP all at a concentration of 1 mg/L in ultra-pure water.

3.3.1.2 Chemicals used for enrichment and extraction experiments

Magnetite (powder, <5 µm, 95%), goethite (powder, 30-63%) and the aluminum oxide Supelclean LC Alumina-A (Brockmann Act. I, particle size 60-325 mesh) were bought from Sigma-Aldrich (Steinheim, Germany). Illite was obtained from The Clay Minerals Society (Chantilly, VA, USA). Sodium phosphate dibasic heptahydrate ($Na_2HPO_4 \cdot (H_2O)_7$)

(≥99%), oxalic acid (≥99%) and sodium hydroxide (≥98%) were purchased from Merck (Darmstadt, Germany). L(+)-Ascorbic acid sodium salt (≥99%) was delivered by Fluka (Buchs, Switzerland).

3.3.1.3 Chemicals for the optimization of separation methods

The following chemicals were used: For **LC-MS1:** aqueous ammonia solution (LC-MS grade, 25%), ammonium acetate from Sigma-Aldrich (Steinheim, Germany) and formic acid (LC-MS grade LiChropur, 98%) from Merck (Darmstadt, Germany). Acetonitrile (LC-MS grade, ≥99%) was purchased from Honeywell (Seelze, Germany). For **LC-MS2**, acetonitrile (ACN)(LC-MS grade, ≥99%) was obtained from Carl Roth (Karlsruhe, Germany), and formic acid (LC-MS grade LiChropur, 98%) from Merck (Darmstadt, Germany). For **CE-MS2**, the same aqueous ammonia solution, ammonium acetate, ACN and formic acid were used as for LC-MS1. Methanol (LC-MS grade, ≥99%) was bought from Fluka (Steinheim, Germany) and acetic acid (LC-MS grade LiChropur, 98%) were both purchased from Merck (Darmstadt, Germany).

3.3.2 Instrumentation

The **CE-MS1** method for quantification of glyphosate from enrichment experiments was conducted as described by Engelbart et al.[44] Bare fused silica capillaries with an inner diameter of 50 µm and a length of 75 cm from Polymicro Technologies (Phoenix, USA) were used throughout the study.

CE-MS2 analysis for all other measurements regarding method development experiments was performed using a 7100 Agilent CE System coupled to a 6150 single quadrupole mass spectrometer (Agilent, Waldbronn, Germany and Santa Clara, CA, USA), the same as for LC-MS1. Bare fused silica capillaries (inner diameter 50 µm) from Polymicro Technologies (Phoenix, USA) were used without further treatment or coated with a double layer using poly(diallyl dimethyl ammonium chloride) (PDADMAC) and polystyrene sulfonate (PSS) by S. Hertweck according to [154]. All capillaries were cut to a length of 75 cm. The electrospray voltage was set to -3.5 kV. The fragmentor voltage was set to 125 V. The sheath liquid was a 1:1 (v/v) mixture of isopropanol and ultrapure water containing 0.01% formic acid at a flow rate of 5 µL/min delivered by an isocratic 1260 infinity pump (Agilent Technologies, Waldbronn, Germany). An aqueous background electrolyte (BGE) of 175 mM formic acid and 40 mM ammonia (adjusted to pH 2.8) and a non-aqueous BGE, prepared by diluting methanol 1:4 with ACN and adding 1 M acetic acid and 25 mM ammonium acetate, were used. Since this BGE is mostly organic, no pH-value was determined. Before use, the outer coating was removed at the outlet of the capillaries using a lighter and the capillary tip was sanded to a 90 ° angle to ensure good conditions at the MS interface. The CE was thermostatted to 25 °C. The capillary was flushed with BGE for 170 s prior to each measurement. Sample injection was performed by applying pressure for a specific time, depending on the experimental setup. After sample injection, a plug of BGE was injected (100 mbar pressure for 10 s) to prevent diffusional losses into the BGE vial. For some measurements, an additional inlet pressure of 50-100 mbar was applied during the CE-MS analysis to reduce analyte migration times and provide stable electrospray conditions. The separation voltage used is specific to the experiments performed.

LC-MS1 analysis for the method development was conducted with an Agilent 1260 Infinity LC system coupled to a 6150 single quadrupole mass spectrometer (Agilent, Waldbronn, Germany and Santa Clara, CA, USA), the same as for CE-MS2. For ionization, an Agilent Jet Stream ion source was used in the negative ionization mode. The nebulizer pressure was set to 35 psi, the drying gas temperature to 250 °C at a flowrate of 11 L/min and the fragmentor voltage to 125 V. Sheath gas was supplied at a flow rate of 11 L/min at a temperature of 325 °C. Single ion monitoring was used for all analytes. Separation was performed using a Raptor Polar X 2.7 µm, 50x2.1 mm column from Restek (Bad Homburg, Germany) connected to a Raptor Polar X, 2.7 µm, 5x2.1 mm EXP guard cartridge. Eluent A consisted of water containing 100 mM ammonium acetate adjusted to pH 8 using aqueous ammonia solution. ACN acidified with 0.1% formic acid was used as eluent B. After injection, the injection needle was dipped into a vial containing a 1:1 mixture of ACN and water to avoid carryover.

Further method development was accomplished using **LC-MS2** with an Agilent UHPLC LC Infinity 1290 coupled to an Agilent QTOF 6548 (Agilent Technologies, Santa Clara, CA). Separation was performed on an Accucore 150 Amide-HILIC-HPLC column, 2.6 µm, 150x2.1 mm from Thermo Fisher Scientific (Schwerte, Germany). An Agilent Jet Stream ion source was used at negative ionization polarity. Between the measurements, the injection needle was cleaned with a 1:1 mixture of Eluent A and Eluent B using the automated LC system. The drying gas temperature was set to 300 °C at a flowrate of 8 L/min, the nebulizer pressure to 40 psi, and the sheath gas flow to 11 L/min with a temperature of 325 °C. The nozzle voltage was 1500 V and the fragmentor voltage was 90-175 V, depending on the experiment. As mobile phases, mixtures of Eluent A containing water acidified with 0.1% or 1% formic acid and Eluent B containing ACN and 0.1% formic acid were used.

3.3.3 Extraction and enrichment experiments of glyphosate

Illite was ground to a fine powder with an Achat mortar and pestle. Prior to the experiment, 6 g of each mineral were washed with 20 mL ultra-pure water by shaking for 30 min at 125 rpm on a shaking table to remove small particles from the minerals. The minerals were then centrifuged for 1 min at 1,520 g. The supernatant was discarded and the remaining mineral was transferred to a petri dish and dried overnight at 50 °C in a ventilated oven. No treatment was performed for Alumina-A.

For the first experiment (Ex1), 1 g each of illite, goethite, magnetite and Alumina-A was weighed into a 50 ml plastic tube and 20 mL of ultrapure-water were added. After shaking at 125 rpm on a shaking table for 30 min, 100 µL of glyphosate solution (1 g/L) were added to the suspension to reach a final concentration of 5 mg/L. The sample was then vortexed thoroughly and placed on a shaking table at 125 rpm for 24 or 48 hours. After centrifugation at 1,520 g for 10 min, the supernatant was removed and the suspension was transferred to a petri dish and dried overnight in a ventilated oven at 50 °C. In general, four different extraction media were used in different combinations. Table 2 summarizes the composition of the extraction media and Table 3 the combinations of extraction media used.

Table 2: Overview of the extraction media used for glyphosate extraction from soil minerals with their abbreviation, composition, concentrations and reference listed.

Abbreviation	Chemicals used	Concentration/ mol/L	Reference
NP	NaOH Na_3PO_4	0.05 0.05	[73]
O	ammonium oxalate oxalic acid	0.23 0.17	[155]
A	ammonium oxalate oxalic acid sodium ascorbate	0.175 0.1 0.19	[152]

In Ex1, the standard extraction procedure involved the addition of either a single extraction medium or the combination of extraction media specified in Table 2 to 80 mg each of illite, goethite, magnetite, or Alumina-A. The total volume of extraction solution/mixture was kept at 250 µL, resulting in a liquid:solid ratio of 25:8. After mixing, the suspensions were sonicated for 30 min and centrifuged at 16,000 g for 15 min. For double extractions, the extraction was repeated with 250 µL of a different medium to address different sorption sites. The supernatants from both extraction steps were combined at a 1:1 ratio. 100 µL of supernatant were acidified with 10 µL of 2 M HCl according to Wimmer et al.,[73] and centrifuged again at 16,000 g. All experiments were performed in duplicates. The isotope labeled internal standard was added prior to measurement using 18 µL of sample and adding 2 µL of G170 (25 mg/L) to reach a final concentration of 2.5 mg/L.

Table 3: List of extraction experiments performed for Experiments 1 and 2 (Ex1, Ex2), with the ratio and abbreviation of the extraction media used (see Table 2). Sample types were low water content mineral suspension (LW) or diluted mineral suspensions, shaken vertically (DV) or horizontally (DH) during glyphosate sorption to the mineral. S denotes standard extraction and D double extraction. The experiments were named using the numbers/letters of each column, thus e.g. Ex1-DV-1:1-NP-A-S as summarized in the last column. Shaking times for Ex1 were 24 h or 48 h and 1 h for Ex2.

	Low water content mineral suspension (LW)/ Diluted mineral suspension vertical (DV) or horizontal (DH)	Ratio of used extraction media	Used extraction media	Standard (S) / Double (D) extraction	Name of experiment
Ex1	DV	1:0	NP	S	Ex1-DV-1:0-NP-S
			A		Ex1-DV-1:0-A-S
			O		Ex1-DV-1:0-O-S
		1:1	NP-A		Ex1-DV-1:1-NP-A-S
			NP-O		Ex1-DV-1:1-NP-O-S
		1:1	NP-A	D	Ex1-DV-1:1-NP-A-D
			NP-O		Ex1-DV-1:1-NP-O-D
			A-NP		Ex1-DV-1:1-A-NP-D
			O-NP		Ex1-DV-1:1-O-NP-D
Ex2	LW	1:1	NP-A	S	Ex2-MI-1:1-NP-A-S
			NP-O		Ex2-MI-1:1-NP-O-S
		1:4	NP-A		Ex2-MI-1:4-NP-A-S
	DV	1:1	NP-A		Ex2-DV-1:1-NP-A-S
			NP-O		Ex2-DV-1:1-NP-O-S
		1:4	NP-A		Ex2-DV-1:4-NP-A-S
	DH	1:1	NP-A		Ex2-DH-1:1-NP-A-S
			NP-O		Ex2-DH-1:1-NP-O-S
		1:4	NP-A		Ex2-DH-1:4-NP-A-S

The influence of the amount of water added during the sorption phase was investigated. (i) For the determination of the recovery, the glyphosate standard and only a small amount of water (liquid:solid ratio of 4) were added to ensure homogenous distribution of spiked analytes on the mineral surface as “low water content mineral suspension” (LW) samples. (ii) To test the suitability of the sample treatment for later enrichment experiments, a larger amount of water (liquid:solid ratio of 20) was used to better mimic environmental samples, e.g. from surface waters, in the following referred to as “diluted mineral suspension”. Furthermore, the impact of mechanical handling on diluted mineral suspensions during the sorption process of enrichment experiments were tested using vertical (DV) and horizontal (DH) positions on the shaking table.

For Experiment 2 (Ex2), 500 mg each of illite, goethite, magnetite, or Alumina-A were weighed into 15 mL plastic tubes, and 2 mL of ultra-pure water (liquid:solid ratio of 4) were added to samples referred to as LW samples and 10 mL (liquid:solid ratio of 20) for DV/DH samples. After shaking the mineral suspension for 30 min, 50 µL of a glyphosate solution (100 mg/L) were added to reach a final concentration of 0.5 mg/L for DV/DH samples and 2.5 mg/L for LW samples. To ensure an equal distribution of the analyte, this was done by submerging the pipette tip into the suspension upon emptying it. Samples were then vortexed thoroughly and placed on a shaking table at 125 rpm for 1 hour. In addition, the supernatant was collected for analysis after centrifugation and prior to drying the precipitate. Extraction of the pellet was performed as described for Ex1, using 50 mg of the mineral phase and 125 µL of extraction medium, resulting in a liquid-solid ratio of 5:2. The LW-samples had a nominal spiked concentration of 10 mg/kg glyphosate when assuming 100% sorption. Prior to measurement, 2 µL of G169 (20 mg/L) were added to 18 µL extract or 2 µL were added to 20 µL of mineral phase supernatant to analyze glyphosate in the supernatant.

3.4 Results and Discussion

In this study, two major aspects in phosphonate analysis were investigated: (i) To improve detection limits for surface water samples, new sorbent materials based on minerals were investigated for the enrichment of glyphosate from surface water samples. With this sample preparation step, a direct analysis of glyphosate by CE-MS would become feasible, reaching the low LODs required for surface water monitoring. (ii) In order to enable studies on the transformation of the APPs DTPMP and EDTMP and their occurrence in environmental samples, new separation methods based on both CE-MS and LC-MS were investigated.

3.4.1 New enrichment method for glyphosate from aqueous samples

Soil minerals would be ideal sorbents given their good availability, low price and high stability. As discussed in the introduction, glyphosate and AMPA were shown to strongly bind to mineral oxides such as goethite, gibbsite, synthetic aluminum oxide and magnetite.[143, 156-158] However, the strong sorption impairs the extraction of glyphosate from the sorbent. Thus, a suitable extraction protocol has to be developed for enrichment or passive sampling. We selected soil minerals from an unpublished previous study based on their recoveries after sequential extraction. Alumina-A (aluminum oxide), illite, goethite, and magnetite were selected due to the high glyphosate recoveries roughly between 82-110% after sequential extraction with NaCl, NaOH and Na_3PO_4 [159] making high extraction efficiencies and thus high enrichment factors likely. For illite, it was demonstrated that after NaCl extraction, 20% and after NaOH extraction more than 90% of glyphosate were recovered,[159] corroborating findings from desorption through coulomb repulsion at a basic pH,[150] but also the presence of rather weak sorption sites. In contrast, both iron oxides, goethite and magnetite showed low recoveries <5% after extraction using NaCl but total recoveries up to 115% for magnetite and 100% for goethite after extraction at a basic pH. After the final extraction using phosphate addition, nearly 120% recovery for magnetite and 115% for goethite were achieved,[159] indicating that sorption sites are present, where the competition with phosphate is vital for extraction, which is in line with literature findings.[143] A similar observation as for the iron oxides was made for Al_2O_3. Furthermore, the minerals are easily available and low at cost.[159]

Besides comparing different minerals, the effects of shaking time of 24 and 48 h during the analyte sorption on the minerals and the composition of the extraction medium were investigated. The extraction media used (see Table 2) were selected for several reasons: It was already shown that the addition of phosphate enhances the alkaline extraction of glyphosate from highly sorptive soils and sediments. High recoveries of 70-90% were reached, due to phosphate acting as an efficient sorption antagonist for glyphosate.[73, 148] In addition, the extraction protocol established by Wimmer et al. is directly compatible with CE-MS analysis after neutralization and centrifugation of the samples.[73] Extraction medium O with oxalate/oxalic acid is commonly used for the determination of oxalate-extractable Fe(II) and Fe(III) in sediments [155] and medium A with ascorbic was used for the determination of trace metals occluded in iron oxides.[152] The main function of oxalate and ascorbic acid is to etch and (partially) dissolve the surface of iron oxide minerals [160, 161] and thus release glyphosate bound to the mineral surface. This is particularly interesting for goethite and magnetite, known to strongly bind glyphosate.[148, 162]

To test the suitability of different minerals to function as a sorbent to enrich glyphosate from aqueous samples such as river water, aqueous suspensions with of 1 g mineral in 20 mL water were spiked with glyphosate at a concentration of 5 mg/L and dried. In Ex1 (see Table 3), 250 µL extraction medium were added to 80 mg of the mineral spiked with glyphosate. If all glyphosate spiked (5 mg/L) had sorbed to the mineral, the concentration would be 100 mg/kg glyphosate. Different extraction media containing NaOH, Na_3PO_4, oxalic and ascorbic acid or mixtures thereof were used (see Table 3). Assuming complete sorption and 100% extraction of the spiked glyphosate from the mineral, the final concentration in the extract of would be 32 mg/L which would indicate an enrichment factor of 6.4. The final extract concentrations and glyphosate recoveries, calculated for both, sorption and extraction for each mineral in Ex1 are shown in Fig. 10A-D.

The aluminum oxide Alumina-A generally revealed concentrations in the extract well below the initial glyphosate concentration of 5 mg/L, ranging from 0.04-1.37 mg/L (see Fig. 10A) leading to enrichment factors <1. The shaking time had no effect for this mineral. The lowest recoveries were found for the medium Ex1-DV-1:0-O-S (see Table 3) (with 0.07 mg/L (24 h) and 0.04 mg/L (48 h) glyphosate) and Ex1-DV-1:0-A-S (with 0.09 mg/L (48 h) glyphosate). The highest concentrations of 1.35 mg/L (24 h) and 1.37 mg/L (48 h) were reached when extracting with basic phosphate medium (Ex1-DV-1:0-NP-S) ions. Still, this concentration would mean that the recoveries were below 5%. Gimsing et al. investigated sorption of different soils from Denmark and demonstrated a strong glyphosate sorption on soils rich in aluminum oxides with a K_d-value of 8.16 L/kg compared to soils containing less aluminum oxides with K_d-values of 4.09-4.19 L/kg.[163] The K_d-value from self-synthesized aluminum-hydroxide gibbsite had an even higher K_d-value with 261 L/kg.[163] We assume that the low recoveries shown in Fig. 10A are due to an insufficient extraction of glyphosate from the mineral rather than incomplete sorption during spiking.

For the clay mineral illite similarly low glyphosate concentrations below 5 mg/L in the extract as for Alumina-A were observed for all extraction media and shaking times, reaching a maximum concentration of 2.32 mg/L for Ex1-DV-1:0-A-S (48 h) (see Fig. 10B). With the combination of Ex1-DV-1:0-O-S, similarly high concentrations in the extract of 2.26 mg/L (24 h) and 2.24 mg/L (48 h) were measured. Considering the recoveries shown in Fig. 10B, the same problem as described for Alumina-A is likely present with illite showing a maximum recovery of 7.3% for Ex1-DV-1:0-A-S (48 h). Gimsing and Borggaard did not see a significant increase in glyphosate sorption on illite when prolonging sorption time to more than 48 h.[143] Even longer shaking times thus cannot be expected to be beneficial for the adsorption nor practical for a later application to environmental samples.

Both iron oxide minerals, goethite and magnetite, revealed glyphosate concentrations in the extract above 5 mg/L (see Fig. 10C and D) and thus an enrichment effect. From the discussion by Gimsing and Borggaard,[143] we can expect a higher sorption capacity of glyphosate on iron oxides than on clay minerals such as illite. The maximum sorption capacity for glyphosate on goethite ranged between 21.8-134.5 mmol/kg and strongly depended on the pH: The maximum sorption capacity was at pH 2 followed by 127.8 mmol/kg at pH 7.[164] The higher sorption capacities can be explained by the pH_{pcz} around pH 8 of goethite. The anionic or zwitterionic glyphosate easily adsorbs on the positively charged mineral surface below its pH_{pcz},[147, 164] whereas at higher pH electrostatic repulsion becomes relevant. Regarding goethite, a $pK_{Langmuir}$-value of 0.62 L/µmol at pH 4 indicates strong glyphosate sorption on goethite.[149] For extraction of glyphosate from goethite, the combination Ex1-DV-1:1-NP-A-S provided the highest concentration reaching 9.96 mg/L (24 h) and thus an enrichment factor of about 2 compared to the original spiking solution. Extraction with Ex1-DV-1:0-NP-S resulted in the second highest concentrations in the extract with 5.84 mg/L (48 h) and 5.50 mg/L (24 h), which are, however, almost equal to the initial spiking concentration, so no enrichment was reached.

The most promising mineral was magnetite in combination with the medium Ex1-DV-1:1-NP-A-S. The concentration reached was 15.86 mg/L (24 h) (enrichment factor of about 3), but also extractions with the combinations Ex1-DV-1:1-NP-O-S (10.99 mg/L (24 h), enrichment factor of about 2) and 1-NP-S (9.26 mg/L (24 h), enrichment factor of almost 2) were promising. Recoveries obtained with Ex1-DV-1:1-NP-A-D or 1:1-NP-O-D were lower and no enrichment was reached with 4.8-6.9 mg/L. In terms of recoveries, the values for goethite and magnetite were the highest for most combinations of extraction media (see Fig. 10C and D).

However, the recoveries were still low, with a maximum of 49.6% for magnetite using Ex1-DV-1:1-NP-A-S. In general, a shorter shaking time during the sorption phase seemed to be beneficial, so spiked mineral suspensions were shaken for 1 h in Ex2 to enhance sorption. For the extraction of glyphosate from iron oxides, the addition of phosphate proved beneficial, corroborating the ability of phosphate to desorb almost 90% of the adsorbed glyphosate from goethite by competitive sorption.[143]

Further, the influence of a change in the order of addition of the extraction media for experiments Ex1-DV-1:1-NP-A-D and Ex1-DV-1:1-NP-O-D compared to Ex1-DV-1:1-A-NP-D and Ex1-DV-1:1-O-NP-D was tested. The idea was to either desorb glyphosate from sorption sites competitive to phosphate sorption or accessible by using high pH and later remove glyphosate from even stronger sorption sites via surface etching by the addition of medium A or O for samples or the other way around for an additional sample set. The strongest effect was observed for magnetite by increasing the glyphosate concentration in the final combined extracts from 3.9 g/L to 6.9 mg/L for sample Ex1-DV-1:1-O-NP-D to -NP-O-D. While this increase is significant with a factor of 1.8, it is still low compared to 11.0 mg/L glyphosate obtained in experiment Ex1-DV-1:1-NP-O-S, especially considering the additional efforts in laboratory work and time required for double extractions. For this reason, no further double extractions were tested.

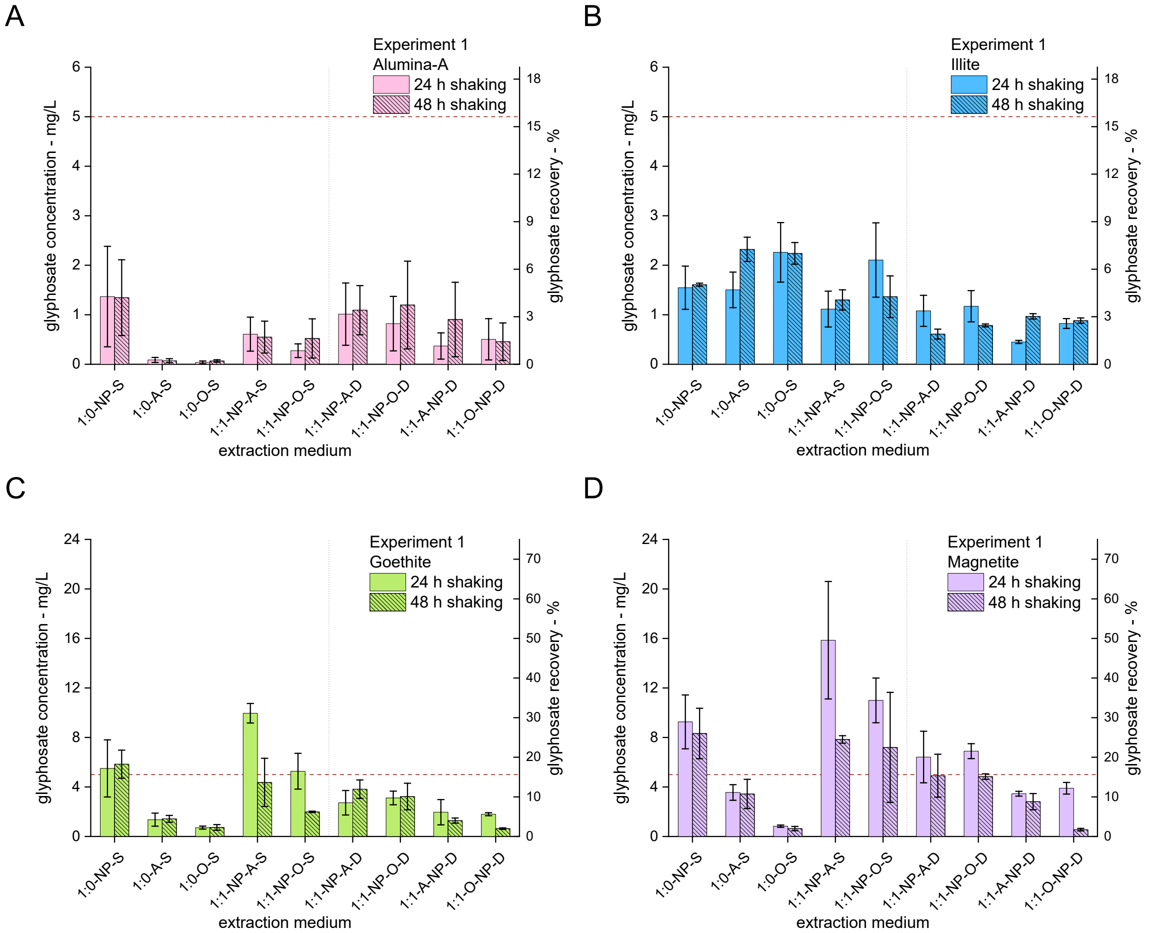


Fig. 10: Results for Ex1: Glyphosate concentrations in the final extract and recoveries from spiking different minerals at a concentration of 5 mg/L reached after extraction using 250 µL of different extraction media or combinations thereof for (A) Alumina-A, (B) illite, (C) goethite and (D) magnetite. Shaking times refer to the sorption phase with glyphosate spiked to the mineral suspension in water. Abbreviations for the extraction media are listed in Table 2, the experiment is summarized in Table 3. The initial spiking concentration of 5 mg/L glyphosate is indicated by a horizontal dashed line. 100% recovery including sorption and extraction would equal 32 mg/L.

To better understand the reason for the low recoveries observed in Ex1 (see Table 3), we first optimized the sorption phase. For the low water content mineral suspension (LW) samples only a small amount of water was used in the suspension for spiking to ensure a homogenous glyphosate distribution and no losses when discarding the supernatant. This enabled the calculation of extraction efficiencies in the extraction phase. For Ex2, a mineral suspension (liquid-solid ratio of 4) was spiked with glyphosate at a concentration of 2.5 mg/L and dried (see Table 3). Briefly, the extraction was conducted in the same manner as for Ex1, adding 250 µL extraction medium to 50 mg of the mineral spiked with glyphosate. If all glyphosate spiked (5 mg/L) had sorbed to the mineral, the concentration would be 10 mg/kg. Promising extraction media containing NaOH, Na_3PO_4, oxalic and ascorbic acid or mixtures thereof were used as in Ex1 (see Table 3). Assuming complete sorption and 100% extraction of the spiked glyphosate from the mineral, the final concentration in the extract of would be 32 mg/L which would indicate an enrichment factor of 6.4 for diluted mineral suspension samples shaken horizontal (DH) or vertical (DV).

The results for Ex2-DV/DH/LW-1:4-NP-A-S, Ex2-DV/DH/LW-1:1-NP-A-S and Ex2-SV/DH/LW-1:1-NP-O-S are shown in Table 4. Alumina-A and goethite showed lowest recoveries in Ex2-LW- samples independent from extraction medium used. For samples Ex2-LW-1:4-NP-A-S, Ex2-LW-1:1-NP-A-S and Ex2-LW-1:1-NP-O-S glyphosate recoveries below 1.5% were determined. Fig. 11A shows the glyphosate recoveries compared to the spiked glyphosate load of 100 mg/kg recoveries. It was determined as the ratio between the concentrations in the extracts versus initial spiked concentration of 2.5 mg/L in this experiment.

Meanwhile, glyphosate recoveries from goethite were higher, especially for sample Ex2-LW-1:4-A-S with the highest glyphosate recovery of 27.6%. As the recoveries for goethite for samples Ex2-LW-1:1-NP-A-S and Ex2-LW-1:1-NP-O-S were 26.4% and 25.8% respectively, the higher portion of A in sample Ex2-LW-1:4-NP-A-S or the presence of ascorbic acid next to oxalic acid (Ex2-LW-1:1-NP-A-S) did not evoke significant differences in recoveries of glyphosate. This is interesting, as the presence of oxalic acid and sometimes ascorbic acid should support extraction via etching of mineral surface, but probably also new sorption sites evolved newly exposed to the solution. In contrary it seems that the effect of pH change by the addition of acids inhibits the desorption by coulomb repulsion, likely given the pH_{pzc} of goethite of 9.3.[165]

The use of the clay mineral illite as a sorbent revealed higher recoveries of glyphosate with a maximum of 50.6% for sample Ex2-LW-1:1-NP-O-S. For this mineral, the addition of ascorbic acid (in experiments Ex2-LW-1:4-NP-A-S and Ex2-LW-1:1-NP-A-S) did not improve the recoveries. Furthermore, the pH_{pzc} for illite is lower than for goethite, so the effect of pH change seems to be less important than for goethite.

By far, best results were obtained for the mineral magnetite, which is also an iron oxide such as goethite. This is interesting, considering the higher K_L-value for goethite if 2.6 compared to 1.1 for magnetite at pH 7 with sorption capacities of 7.9 mg/g and 6.7 mg/g, respectively.[165] In contrast, the reported pH_{pzc} for magnetite is clearly lower with 5.3 compared to 9.3 for goethite,[165] which can explain the higher extraction efficiencies obtained with less basic extraction media used. Highest extraction efficiencies were obtained for Ex2-LW-1:4-NP-A-S and Ex2-LW-1:4-NP-O-S with 64.9% and 63.7%, respectively. The increase of the A portion in Ex2-LW-1:4-NP-A-S compared to Ex2-LW-1:1-NP-A-S seems to be beneficial increasing recoveries from 56.4% to 64.9%. But still, the extraction efficiency stayed below 70%.

Table 4: Glyphosate recoveries of sorption and extraction experiments from the minerals goethite, illite, magnetite and Alumina-A using the different extraction media listed in Table 2. Experimental details are described in Section 3.3.3 and the sample handling and extraction media combination chosen are listed in Table 3. The shaking time during the sorption phase with glyphosate spiked to the mineral suspension in water was 1 h.

Mineral used	Experiment name		Glyphosate recovery/ %
Goethite	Ex2-LW-	1:4-NP-A-S	27.6
		1:1-NP-A-S	26.4
		1:1-NP-O-S	25.8
	Ex2-DV-	1:4-NP-A-S	11.9
		1:1-NP-A-S	22.2
		1:1-NP-O-S	19.4
	Ex2-DH-	1:4-NP-A-S	15.5
		1:1-NP-A-S	29.1
		1:1-NP-O-S	16.8
Illite	Ex2-LW-	1:4-NP-A-S	44.2
		1:1-NP-A-S	28.3
		1:1-NP-O-S	50.6
	Ex2-DV-	1:4-NP-A-S	6.4
		1:1-NP-A-S	4.4
		1:1-NP-O-S	6.4
	Ex2-SH-	1:4-NP-A-S	6.3
		1:1-NP-A-S	5.0
		1:1-NP-O-S	7.4
Magnetite	Ex2-LW-	1:4-NP-A-S	64.9
		1:1-NP-A-S	56.4
		1:1-NP-O-S	63.7
	Ex2-DV-	1:4-NP-A-S	-
		1:1-NP-A-S	66.3
		1:1-NP-O-S	37.7
	Ex2-DH-	1:4-NP-A-S	48.6
		1:1-NP-A-S	46.8
		1:1-NP-O-S	51.6
Alumina-A	Ex2-LW-	1:4-NP-A-S	0.0
		1:1-NP-A-S	1.5
		1:1-NP-O-S	0.6
	Ex2-DV-	1:4-NP-A-S	0.0
		1:1-NP-A-S	0.2
		1:1-NP-O-S	0.8
	Ex2-DH-	1:4-NP-A-S	0.5
		1:1-NP-A-S	0.9
		1:1-NP-O-S	0.4

In contrast to the LW experiments, later applications will deal with sorption in diluted suspensions, so a higher volume of water was used during spiking to better mimic environmental samples. As there was no overhead shaker available for this study, the influence of shaking geometry on the shaking table for diluted suspended mineral samples (DV = vertical and DH = horizontal during sorption) was investigated (results see Table 4). Assuming complete sorption after spiking the mineral at a glyphosate concentration of 0.5 mg/L and 100% extraction of the spiked glyphosate from the mineral, the final concentration in the extract of would be 4 mg/L which would indicate an enrichment factor of 8. In this the experiment, we also quantified glyphosate in the supernatant of diluted mineral samples DH and DV to better understand losses during the sorption phase. Fig. 11A shows the glyphosate recoveries when comparing the concentrations in the extracts to the initial spiked concentration of 0.5 mg/L in this experiment. Fig. 11B shows the fraction of glyphosate detected in the supernatant after sorption.

No glyphosate was found in the supernatant of goethite and magnetite demonstrating a sufficient sorption capacity (see Fig. 11A). For Alumina-A, only 2.5% of the spiked glyphosate was still present in the supernatant of sample Ex2-DV after 1 h of shaking for the samples in tubes shaken vertically, while no glyphosate was detected in the supernatant of the samples DH with a more rigorous shaking in tubes placed horizontally on the shaker. For illite, only a small difference was observed between the shaking modes, but most of the glyphosate remained in the supernatant (106.4% for E2-DV sample and 96.3% for Ex2-DH sample) corroborating the results of the series in Ex1 where the low recoveries for illite were ascribed to a too low sorption. For illite, adsorption was discussed to be dominated by ligand exchange with the hydroxyl groups exposed at the edges of the mineral, as no complexes were formed with the interlayer cation K^+.[143] This is further corroborated by the higher recoveries obtained when extracting low water content diluted mineral illite (see Fig. 11B) compared to the diluted suspensions, where non-sorbed glyphosate was discarded with the supernatant. In contrast, the limited recoveries for Alumina-A seem to be caused by challenges during the extraction, which must have been insufficient indicating a very strong sorption of glyphosate on alumina. [143] Since both Alumina-A and illite showed insufficient recoveries due to strong sorption of glyphosate for Alumina-A but too weak sorption for illite, none of these two minerals are suitable for enrichment procedures. In contrast, the iron oxides were promising, which was further corroborated by comparing the sorption capacity of glyphosate on goethite reported to be 125 mmol/kg [147] vs. only 5.2 mmol/kg on illite.[143].

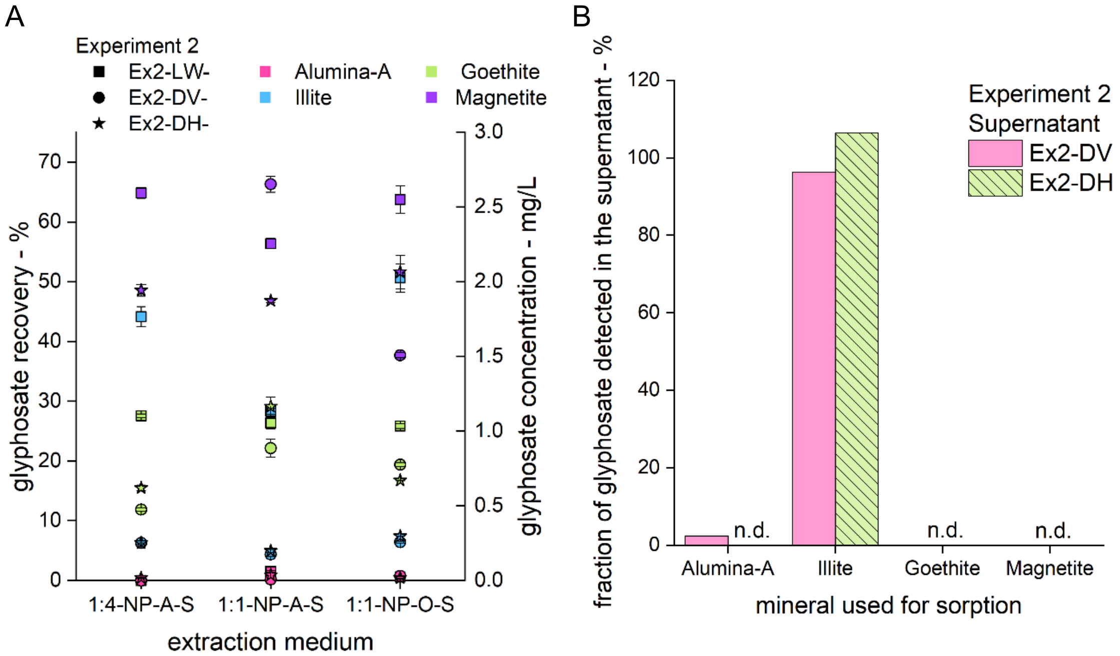


Fig. 11: Results for Ex2: (A) Glyphosate concentrations in the final extract and recoveries after sorption and extraction using promising combinations of extraction media for Alumina-A, illite, goethite and magnetite. (B) Fraction of glyphosate recovered in the supernatant of diluted mineral suspension after shaking for 1 h relative to the initial glyphosate spiking concentration of 0.5 mg/L. Extraction media abbreviations are listed in Table 2. Diluted mineral suspensions were shaken vertically (Ex2-DV) or horizontally (Ex2-DH). The initial spiking concentration was 0.5 mg/L glyphosate for Ex2-DH- and Ex2-DV- and 2.5 mg/L for low water content mineral suspension (Ex2-LW-). For details on sample preparation see Section 3.3.3 and for an overview of experiments Table 3. During the sorption phase, the mineral suspensions in waste with glyphosate spiked were shaken for 1 h.

Among the iron oxides, goethite showed lower to similar recoveries for glyphosate for Ex2-DH-1:1-NP-A-S (29.1%), Ex2-DV-1:1-NP-A-S (22.2%) and even Ex2-LW-1:1-NP-A-S (26.4%) than in Ex1-DV-1:1-NP-A-S (31.1% after 24 h shaking). Clearly, a reduction in shaking time does not negatively impact the recoveries, which is consistent with the literature indicating a fast and complete sorption of glyphosate in goethite within about 5 h.[143, 148]

Again, the most promising results were obtained for magnetite. For this mineral, the prolongation of the sorption phase was important with an increase in recoveries by a factor of about 2 using the same extraction medium (Ex1-DV-1:1-NP-A-S (34.1%, 24 h shaking) vs. Ex2-DV-1:1-NP-A-S (66.3%)). Furthermore, Ex2-DH-1:1-NP-O-S (63.7%) also showed a comparable high recovery to Ex2-DV-1:1-NP-A-S (66.3%). Interestingly, samples Ex2-DR and Ex2-DV both extracted with a basic medium with phosphate and oxalic acid (1:1-NP-O-S) showed a large difference between low water content mineral suspension (63.7%) vs. diluted (37.7%) mineral suspensions (DV)(Ex2-DV-1:1-NP-O-S vs.Ex2-LW-1:1-NP-O-S), especially considering that no glyphosate was found in the supernatant of the diluted mineral suspension samples.

Now, comparing the differences magnetite of the sample preparation (LW/ DV/ DH) no clear trend can be observed (see Table 5). For samples using 1:1-NP-A-S as extraction medium (Ex2-LW-1:1-NP-A-S, Ex2-DV-1:1-NP-A-S, Ex2-DH-1:1-NP-A-S, details in Table 3) recoveries were as follows DV>LW>DH. Contrary, for samples extracted with 1:1-NP-O-S (Ex2-LW-1:1-NP-A-S, Ex2-DV-1:1-NP-A-S, Ex2-DH-1:1-NP-A-S, details in Table 3) order was different with LW>DH>DV. This cannot be explained at the moment because recovery for LW samples was expected to be the highest for all extractions due to directly spiking of glyphosate into the mineral suspension compared to DH and DV samples. The further discussion of this topic can be omitted by the use of an overhead shaker, which guarantees complete mixing of the mineral suspensions during sorption.

Table 5: Glyphosate recoveries of Ex2 sorted by sample preparation (LW/DV/DH) and extraction media used (1:1-NP-A-S or 1:1-NP-O-S). Experimental details are described in Section 3.3.3 and all experiments conducted in Table 3.

	Glyphosate recovery/ %	
	1:1-NP-A-S	1:1-NP-O-S
Ex2-LW-	56.4	63.7
Ex2-DV-	66.3	37.7
Ex2-DH	46.8	51.6

Overall, Ex1 and 2 showed that magnetite is clearly the most interesting mineral to be used as a sorbent for the enrichment of glyphosate from aqueous samples. In Ex2, an enrichment factor of 5 was achieved. This is still low compared to the enrichment factors of 2-500 reported in literature for various analytical methods coupled to LC-MS using SPE columns with reversed phase or ion exchange sorbents or using molecular imprinted polymer cartridges, partially combined with evaporation or lyophilization and resuspension for enrichment.[8, 133, 135, 166-173] An enrichment factor as high as 1000 was reached using 500 mL of filtered marine water after electrodialysis and sorption via molecular imprinted polymers.[174] The enrichment of glyphosate can further be enhanced using large sample volumes. Sample handling can further be facilitated given the magnetic property of magnetite, which allows to collect the mineral phase by magnets as has already been implemented for numerous (biochemical) applications. Magnetite nanoparticles [162] or coated magnetite nanoparticles [175, 176] are already used for the removal of glyphosate from water in remediation applications and may be interesting to be used as a sorbent. It is important to note that it is likely possible to also enrich AMPA as a similar sorption behavior as for glyphosate was observed on magnetite.[177] In addition, glyphosate enrichment by using magnetite is considerably less expensive compared to the aforementioned methods.

3.4.2 Separation of aminopolyphosphonates and their possible transformation products

Electrophoresis

Shamsi and Danielson [53] used CE with indirect photometric detection for the analysis of the polyphosphonates etidronic acid, ATMP, DTPMP, EDTMP but a transfer of this method to CE-MS is not possible due to the use of non-volatile boric acid/tetraborate in their BGE which also contained adenosine monophosphate, presumably to reduce analyte interaction with the capillary surface due to competition with the phosphate for sorption sites. A similar approach was followed by Ortega-Gadea et al. using trichloroacetic acid [66] and Bernabé-Zafón et al. using adenosine triphosphate [67] in their BGEs combined indirect photometric detection and MS-detection with the aim to characterize industrial and technical grade polyphosphonates such as DTPMP. The method published by Bernabé-Zafón et al. was applied for quality control of industrial phosphonates but concentrations used for method development were rather high with e.g. 2 g/L DTPMP,[67] not stating LODs.

However, since CE-MS was proven to be highly suitable for the analysis of small phosphonates such as glyphosate and AMPA,[39, 70, 73] we assumed that also similarly structured APPs and transformation products thereof could be analyzed with the method developed. This would be interesting as the CE-MS method was able to routinely analyze glyphosate and AMPA in extracts of soil and sediment samples.[73] We tested our CE-MS method with its acidic BGE (made of 175 mM formic acid and 40 mM NH_3) with a pH of 2.8 using a bare fused silica capillary [73] for the analysis of a broad range of commercially available amino(poly)phosphonates including DTPMP, EDTMP, glyphosate, AMPA, IDMP, *N*-acetyl-AMPA, *N*-acetyl-glyphosate, *N*-methyl-AMPA and glufosinate. A CE-MS electropherogram with the extracted ion electropherograms of the smaller aminophosphonates is shown in Fig. 12A. Sharp signals were obtained for all mono- and diphosphonate analytes from analyte mixture. No separation between *N*-acetyl-glyphosate (7.0 min) and *N*-acetyl-AMPA (7.0 min) and also AMPA (13.1 min) and *N*-methyl-AMPA (13.0 min) was achieved necessitating a further optimization of the separation selectivity. While the analysis of all smaller analytes was straightforward, the analysis of larger the analytes DTPMP and EDTMP was strongly impaired by their interaction with the surface of the bare fused silica wall. This strong interaction is presumed to be due to a strong interaction of the phosphonate moieties similar to their sorption to mineral oxides e.g. clay mineral.[148] With the high number of phosphonates moieties in DTPMP and EDTMP, sorption problems on the capillary surface are more likely than for mono- or diphosphonates (e.g. IDMP). Fig. 12B exemplarily shows electropherograms for DTPMP injected at different concentrations of 0.2-0.6 mg/L. Only, at a high concentration of 0.6 g/L DTPMP, a sharp analyte signal was observed, otherwise extremely broad “signals” covering several minutes were detected. This indicates that DTPMP self-coats the capillary surface, paving the way for excess DTPMP to migrate through the capillary when the concentration is sufficiently high. This hypothesis is further corroborated by acceptable peak shapes in CE methods using phosphates or phosphonates in the BGE, e.g. adenosine triphosphate,[67, 178] phenylphosphonic acid [179] or ribonucleotides with phosphate groups such as uridine monosphosphate.[53] It is likely that these additive occupy relevant sorption sites on the capillary wall due to similar interactions.

To reduce sorption to the capillary surface and reduce the electroosmotic flow (EOF) to increase resolution, 20 vol% MeOH were added to the BGE at pH 2.8 (175 mM formic acid, 40 mM ammonia). Only minor changes in the selectivity (see electropherogram in

Fig. 12C) compared to the standard CE-MS method (see electropherogram in Fig. 12A) were reached. *N*-acetyl-compounds still comigrated (6.4 min) and AMPA and its methylated derivative were not baseline-separated (11.1 min and 10.9 min). In general, migration times were shorter for all analytes, as expected with a weaker EOF. No signal was detected for the two large APPs EDTMP and DTPMP.

Since adding an organic modifier did not improve the separation, a fully non-aqueous BGE was investigated using the aprotic solvent acetonitrile in combination with methanol. The BGE consisted of 75 vol% MeOH, 25 vol% ACN, containing 25 M ammonium acetate and 1 M acetic acid, similar to many applications in nonaqueous capillary electrophoresis.[180] The sample was the mixture of analytes in water, keeping in mind that environmental samples would mostly be aqueous extracts of sediments or activated sludge. As shown in the electropherogram (see Fig. 12D), no significant improvement in the resolution was observed and even a loss in separation efficiency with broader peaks for the small aminophosphonates. The critical peak pairs with *N*-acetylated compounds and *N*-methyl AMPA remained. In addition, no DTPMP or EDTMP were detected in these experiments, even when applying a high internal pressure of 100 mbar applied during the analysis to assure the transport of all phosphonates to the MS.

To inhibit the EOF without the addition of organic modifiers to the BGE, the capillary was coated with a double layer coating using PDADMAC and PSS to reverse the EOF and to reduce the chance for hydrogen bonding. Nevertheless, as shown in the electropherogram in Fig. 12E, the resolution was clearly inferior an no DTPMP or EDTMP was detectable, even at a concentration of 1 mg/L.

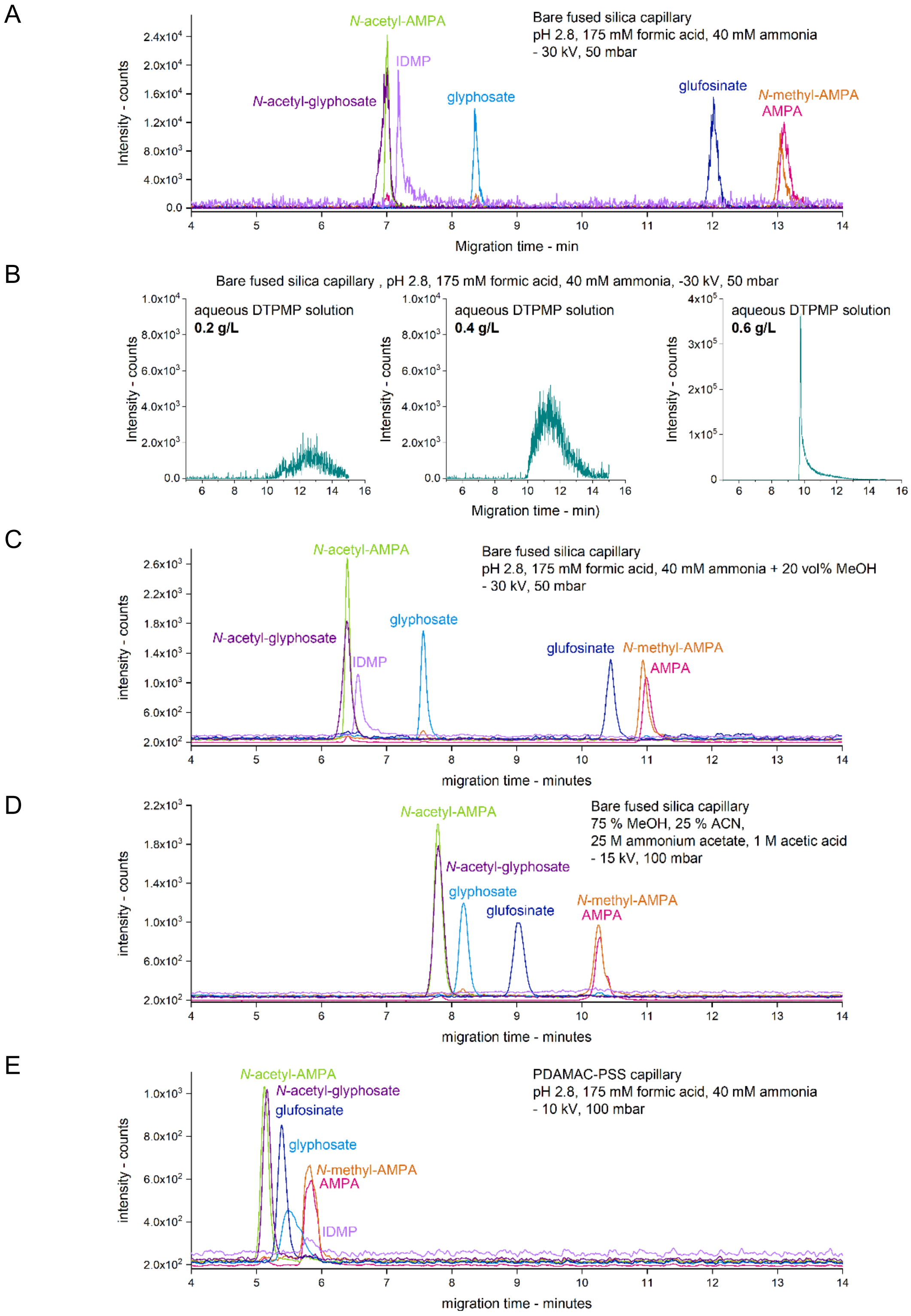


Fig. 12: Electropherograms of the analyte mixture at a concentration of 0.5 mg/L (A) and 1 mg/L (C-E) for each component and aqueous DTPMP solutions at concentrations of 0.2-0.6 mg/L (B). All capillaries for (A) and (C-E) were 75 cm in length, capillary for (B) had a length of 100 cm. Bare fused silica capillaries (A-D) and a capillary with a PDADMAC-PSS-double layer coating (E) were used. Injection was performed by applying 100 mbar for 15 s (A-D) or 75 mbar for 15 s (E), followed by a plug of BGE by applying 100 mbar for 10 s. The following BGEs were used: (A, B, E) aqueous 175 mM formic acid and 40 mM NH_3 adjusted to pH 2.8; (C) the BGE used for (A) and (B) was diluted with 20 vol% MeOH; (D) a nonaqueous BGE made of 75 vol% MeOH, 25 vol% ACN, containing 25 M NH_4OAc and 1 M acetic acid. For (A), (B) and (C), the separation voltage was set to -30 kV with a voltage ramp from -15 kV to -30 kV within the first 1.5 min with an internal pressure of 50 mbar to overcome the EOF. For (D), the separation voltage was -15 kV and for (E) -10 kV, both with an internal pressure of 100 mbar. (A) and (B) were obtained using CE-MS1 and (C-E) using CE-MS2.

While the original CE-MS method optimized for glyphosate and AMPA analysis proved also applicable to small mono- and diphosphonates, the APPs EDTMP and DTPMP could not be analyzed by CE-MS with the buffer systems tested. The method was applied to extracts of sediments impacted by wastewater. Not only glyphosate and AMPA but also IDMP and for the first time, *N*-methyl-AMPA were detected in a sediment extract from a sampling site directly at the point of discharge of the WWTP Heroldsberg in the receiving river Gründlach (see Fig. 13, detailed description of the WWTP Heroldsberg in Section 5.3.2 and of the extraction method in Section 5.3.3) demonstrating the applicability of the CE-method and its sufficient matrix tolerance. *N*-methyl-AMPA has first been described as a transformation product of DTPMP after incubation with cyanobacteria cultures.[181] In a previous study the APP transformation product IDMP was detected by Nowack et al. in WWTP influents,[38] but as sediments of the receiving waters were not investigated, this study is the first one to detect it in receiving water sediment to our knowledge.

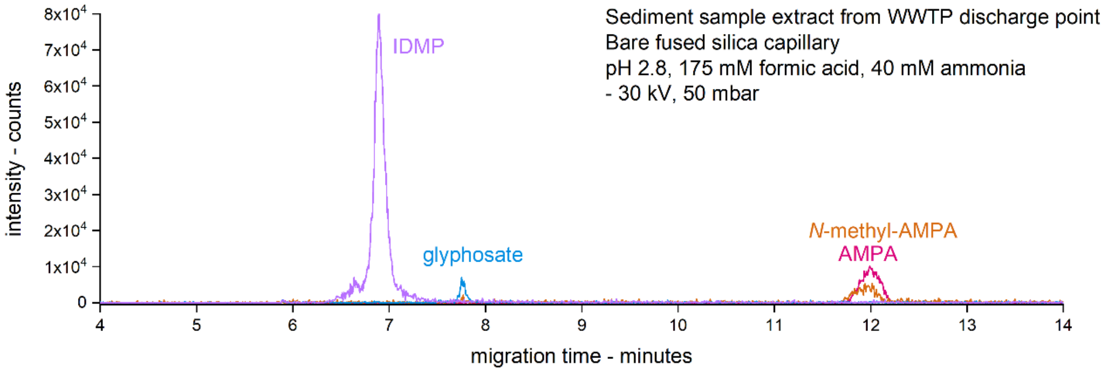


Fig. 13: Electropherogram of a sediment extract from a sampling site in the receiving river Gründlach directly at the discharge of the WWTP Heroldsberg (detailed description of WWTP Heroldsberg in Section 5.3.2, extraction method in Section 5.3.3). Separation conditions were the same as described for Fig. 12A. CE and MS instrumentation as described in Engelbart et al. were used.[44]

Chromatography

Chromatography was investigated as an alternative to CE-MS to also cover the large APPs DTPMP and EDTMP. A previously published HILIC method for the analysis of EDTMP and its possible phototransformation products such as IDMP and AMPA using a SeQuant ZIC-HILIC column (150 × 2.1 mm, 3.5 µm) [63] could not be reproduced (data not shown). Furthermore, the runtime of 45 min seemed too long as it necessitated a high consumption of organic solvents. The European Union Reference Laboratory lists the column Raptor Polar X for the separation of a wide range of polar pesticides including such glyphosate.[182] It was further investigated in this study as it provides mixed-mode separations with both HILIC and IC separation modes. The relevance of these modes can be tuned by the amount of organic solvent used in the mobile phase. Preliminary experiments with this column only using water and ACN as eluents, both acidified with 1-5% formic acid, failed to detect the two larger APPs DTPMP and EDTMP (data not shown). Thus, the ionic strength and pH of the eluent were modified using 100 mM ammonium acetate as buffering component for the aqueous phase in Eluent A, while ACN was acidified with 0.1% formic acid and used as Eluent B, as recommended in a technical guide.[183] Glufosinate was the only analyte well separated, whereas all other analytes coeluted over a range of 11-16 min (see Fig. 14A) with very poor separation efficiencies. Particularly, for glyphosate and IDMP very broad peaks appeared, with a peak width for

IDMP of nearly 5 min. Clearly, the Raptor Polar X column was not suitable for the analysis of the larger APPs and performed poorly for many smaller phosphonates.

Next, two different HILIC columns, Waters BEH Amid and Accucore 150 Amid, were tested for their ability to separate the phosphonates in the analyte mixture (see Fig. 14). The Waters BEH Amid column uses ethylene-bridged-particles with a trifunctionally bonded amide phase which reduces the formation of Schiff bases with analytes, compared to an amine phase and is stable from pH 2-12. The Accucore 150 Amid column also uses an amid phase for separation, but bound to solid core particles and is stable over a pH range of 2-9. Both columns are commonly used for the separation of hydrophilic molecules such as metformin [184] or glycans,[185-187] but also amino acids.[188, 189] As the Acquity UPLC BEH Amide column is also listed in the compendium published by the European Union Reference Laboratory as suitable for the analysis of glyphosate and glufosinate,[182], the column seemed promising to cover further small transformation products but also the parents DTPMP and EDTMP. The chromatogram shown in Fig. 14B, obtained using the BEH Amid column, shows multiple peaks for AMPA and *N*-methyl-AMPA. The peaks at a retention time of 1.7 min can be explained by in source fragmentation of *N*-acetyl-AMPA to AMPA, but the origin of another peak with identical m/z remains to be elucidated. The diphosphonate IDMP (peak at 2.6 min) showed a broad signal of low intensity. *N*-acetyl-glyphosate and *N*-acetyl-AMPA almost coeluted with retention times of 1.7 min and 1.8 min. EDTMP and DTPMP were not detectable using different gradients on this column.

With the Accucore Amid HILIC column, signals for EDTMP and DTPMP were obtained (see Fig. 14C and D), although still at low intensities given the high concentration of the injection solution of 5 mg/L. In the isocratic measurement with 45% eluent A (see Fig. 14C), the overall separation time was very short with 4 min. Although a signal was detectable for all analytes, the separation efficiency and resolution were insufficient. Therefore, the flow rate was reduced from 0.3 mL/min to 0.25 mL/min to increase the separation time.

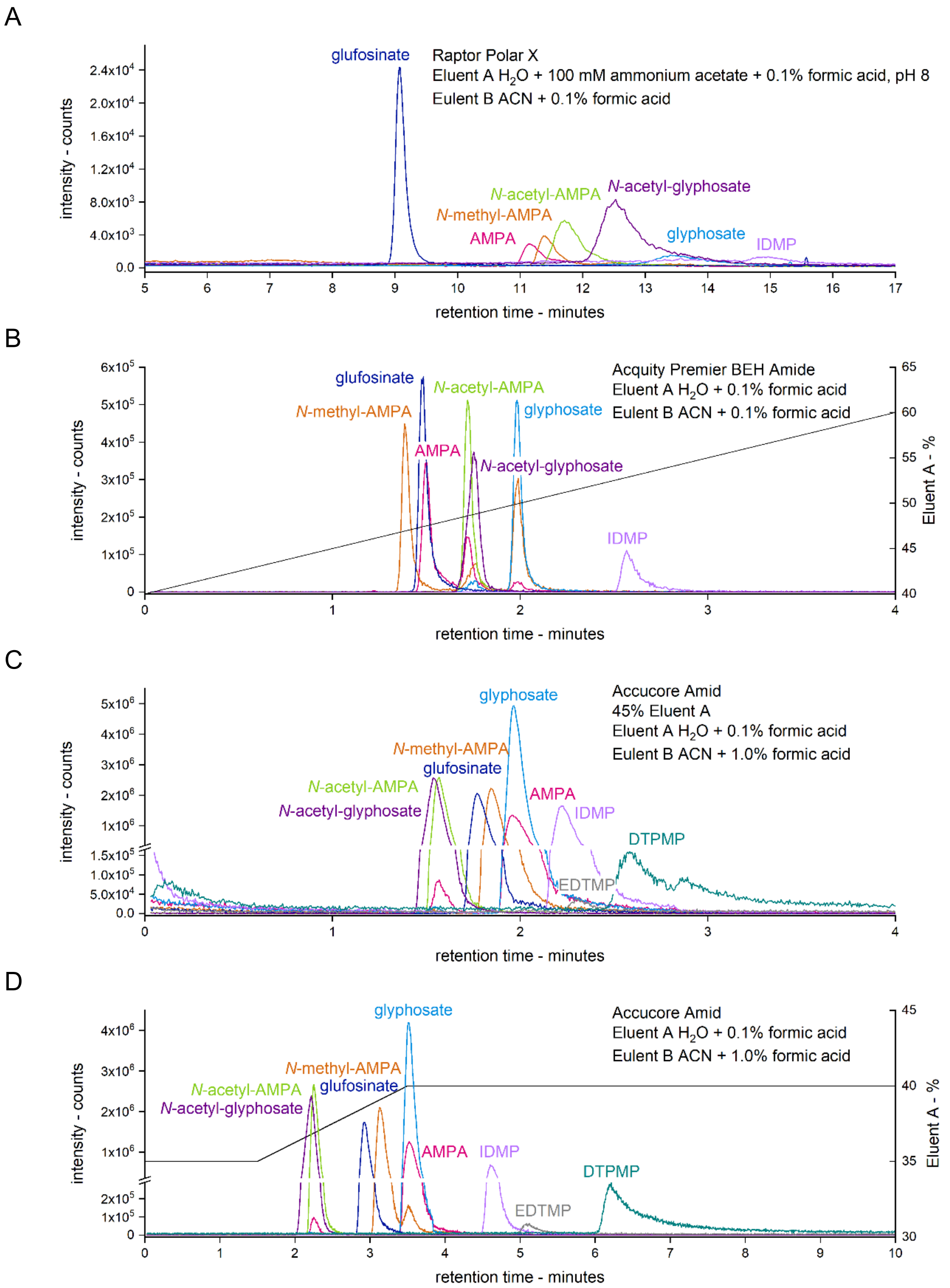


Fig. 14: Chromatograms of analyte mixture obtained using LC-MS1 equipped with a Raptor Polar X column (A) and LC-MS 2 equipped with a Waters BEH amid column (B) and an Accucore Amid column (C, D). Concentrations were 1 mg/L diluted 1:1 with ACN for (A) and 5 mg/L diluted 1:5 with ACN for (B-D). The injection volume was 5 µL and the column temperature was 35 °C for measurement (A) and 30 °C for measurements (B-D). Eluent A was water containing 100 mM ammonium acetate and 0.1% formic acid adjusted to a pH of 8 using ammonia for (A), 0.1% formic acid (B-D); and Eluent B was ACN containing 0.1% formic acid (A, B) or 1% formic acid (C, D). For (A) gradient was as follows: hold 10% A for 2 min, linear ramp to 90% A over 8 in, hold for 5 min. The gradient for (B) was: 40% Eluent A increasing to 75% in a linear ramp over 7 min. (C) was an isocratic measurement with 45% Eluent A. (D) started with 35% Eluent A for 1.5 min. which was increased to 40% Eluent A within 2 min. The flow rate was 0.35 mL/min (A, B), 0.3 mL/min (C) and 0.25 mL/min (D). Mass spectrometric settings were: fragmentor voltage 125 V for (B), but it was reduced to 90 V at the beginning of the separation to reduce in-source fragmentation of glyphosate, and then back to 125 V after 2.85 min. (C, D).

In order to more strongly retain the analytes on the column and further improve resolution and separation efficiency, a gradient elution was made with the lower flow rate of 0.25 mL/min (see Fig. 14D), starting with 35% Eluent A for 1.5 min. and then changing to 40% Eluent A within 2 min. The desired effect was achieved and the analysis time reached 8 min. In addition, the peak shape of both DTPMP and EDTMP was improved, resulting in a higher signal intensity. Compared to the isocratic conditions in Fig. 14C, the separation efficiency also improved. Under the conditions tested, AMPA (3.5 min) and glyphosate (3.5 min), *N*-acetyl-glyphosate (2.2 min) and *N*-acetyl-AMPA (2.3 min) coeluted. However, since this was the only column able to cover all analytes from the phosphonate mixture, further method development with this column is intended to achieve a separation of all analytes. Further mobile phases have to be investigated to increase the selectivity, e.g. adapting the pH, which should change the retention especially of the smaller aminomonophosphonates, such as glyphosate, AMPA and *N*-acetyl-glyphosate and -AMPA, with their first pK$_a$ values ranging between pH 1.14 and 5.51.[190] Beside the use of eluents with a higher pH, a higher ionic strength could provide a better separation efficiency for the largest APPs tested by reducing ionic interactions.

As a last chromatographic method, we investigated the method published by Wang et al.,[65] who analyzed the intact APPs in wastewater after derivatization with trimethylsilyl diazomethane (structures of native and methylated DTPMP see Fig. 15A). The method was slightly modified allowing the reaction mixture to evaporate hexane present in the commercial solution of trimethylsilyldiazomethane, which impaired chromatographic separation. In addition, the fraction of the organic solvent in the mobile phase was increased by changing the gradient. The method was combined with a sample preparation for sediment samples, where APPs were extracted using nitrilotriacetic acid at a basic pH with a following ion exchange step.[52] A chromatogram of an aqueous standard and a sediment sample from the river Gründlach near the discharge point the WWTP Heroldsberg is shown in Fig. 15B. Unfortunately, as also reported by Wang et al. [65], quantification of DTPMP was difficult and necessitated standard addition.

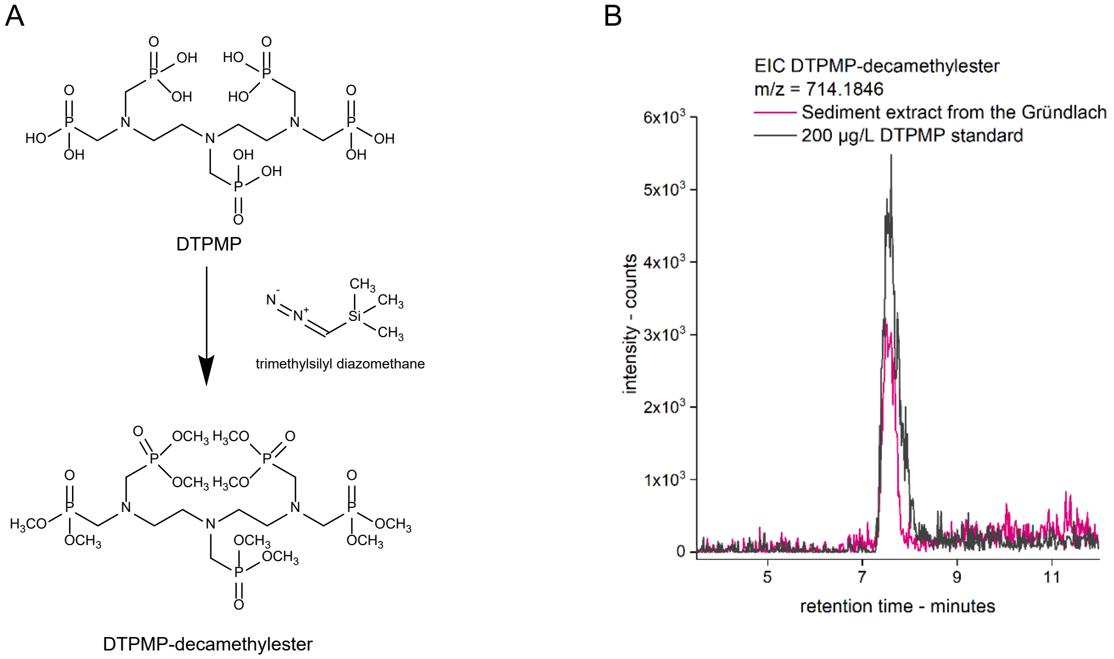


Fig. 15: (A): Chemical structures of native and methylated DTPMP after derivatization using trimethylsilyldiazomethane according to Wang et al.[65] (B): Chromatograms of aqueous DTPMP standard (200 µg/L) and sediment extract after derivatization using the derivatization method adapted from Wang et al.[65] and extraction adapted from Armbruster et al.[52] Further experimental details for sediment sample are described in Section 5.3.2 and 5.3.3.

3.5 Conclusion

This study clearly demonstrates that using a soil mineral as a sorbent, glyphosate can be enriched from aqueous samples and analyzed by CE-MS. This combination provides the fast analysis of glyphosate in surface water samples as a direct analysis without derivatization is possible and may serve as an alternative to classical chromatographic techniques and derivatization. This study showed that concentrations in the final extract for both illite and Alumina-A were clearly below the initial spiking concentration of 5 mg/L glyphosate, so that no enrichment was reached (see Table 3 and Section 3.3.3 for experimental details). For Alumina-A, the sorption was very strong and extraction was insufficient. Illite was not suitable for extraction because the sorption was too weak. In contrast, both iron oxides investigated, magnetite and goethite showed concentrations above the initial concentration, with higher or similar concentrations and even with a shorter sorption time of 24 h compared to 48 h. For magnetite, shorter sorption times were clearly beneficial reaching recoveries of 66.3% and thus an enrichment by a factor of 5. Finally, magnetite and goethite performed well regarding both sorption and extraction but with recoveries in favor of magnetite.

The optimization of the extraction conditions was vital given the strong sorption of phosphonates to soil minerals. The addition of a competitor for sorption sites such as phosphate proved to be necessary for a successful extraction. The implementation of a double extraction did not improve enrichment factors, as recoveries detected were lower than those using the standard extraction method when using the same extraction medium in both steps. The addition of compounds etching the mineral surface such as ascorbic and oxalic acid had a beneficial effect, resulting in glyphosate concentrations higher by a factor of 1.8 for magnetite (comparing Ex1-DV-1:0-NP-S (9.3 mg/L) and Ex1-DV-1:1-NP-A-S (15.9 mg/L)).

In-depth optimization of extraction conditions using magnetite revealed the highest potential for successful glyphosate enrichment from surface water samples. The highest enrichment factor achieved was about 5 using magnetite when combining phosphate with ascorbic or oxalic acid (Ex2-LW-1:4-NP-A-S, Ex2-DV-1:1-NP-A-S and Ex2-LW-1:1-NP-O-S). This result forms a promising starting point for further enrichment experiments using magnetite as a sorbent. Matrix effects of environmental samples still need to be considered, but are not expected to prevent enrichment due to the strong sorption of glyphosate on magnetite. By increasing the liquid:solid ratio and using magnets to collect magnetite after sorption to omit mineral drying in the protocol, enrichment factors can be expected to strongly increase and sample preparation time to strongly decrease. Furthermore, the modification of the extraction medium used for routine analysis in our laboratory [73] was improved in these experiments by etching the surface upon the addition of ascorbic acid and oxalate. In the future, we investigate if other phosphonates can also be enriched with the strategy proposed for glyphosate.

This is in line with the broadening of analytical techniques to quantify larger APPs such as DTPMP and EDTMP together with their transformation products preferably within one method. Ideally, derivatization-free direct methods are used to account for the different number of phosphonate groups and amine moieties applicable for derivatization.

An adaptation of our previous CE-MS method for glyphosate analysis using the standard BGE at low pH [70] was possible for the smaller phosphonates with one or two phosphonate moieties. However, neither adding organic solvents nor using a non-aqueous BGE allowed to detect DTPMP or EDTMP. Only at extremely high concentrations, signals for these compounds were observed but with unsatisfactory peak shapes (see Fig. 12B) due to the strong sorption to the capillary surface. The use of a negatively charged coating neither improved the separation for smaller transformation products nor allowed the detection of DTPMP and EDTMP (see Fig. 12E). Future work will focus on other surface coatings or BGE modifications such as the implementation of a phosphate or phosphonate surface, adapting the methods using e.g. adenosine-triphosphate in the BGE as shown in previous studies [67] to reduce the formation of hydrogen bonds and inhibit sorption of phosphonate analytes. Ideally, a phosphonic acid coating can be implemented as achieved with a dynamic coating using phenylphosphonic acid [191], albeit MS compatibility will be challenging.

In parallel, chromatographic separations with different HILIC stationary phases were tested for the separation of transformation products of DTPMP and EDTMP and the substances themselves. DTPMP and EDTMP could not be detected using a Raptor Polar X or Acquity BEH Amid column under different separation conditions (see Fig. 14A and B). The most promising results for the separation were achieved using the Accucore 150 Amid column, finally achieving the elution of all analytes of interest. The separation efficiencies for IDMP, EDTMP and DTPMP with their higher number of phosphonate groups were still low (see Fig. 14C, D, E), but only eluents acidified with formic acid were tested. The change of eluent pH and the variation of the ionic strength of the mobile phase will surely further improve separation in future experiments. As the methods investigated are not yet suitable for the analysis of environmental samples for all analytes at the same time, the method published by Wang et al. [65] was successfully implemented after some adaptations, albeit the method requires derivatization. This method enabled the quantification of DTPMP and was used in combination with the routine CE-MS analysis to cover the analysis of transformation products. This combination allowed the determine the phosphonates in sediment samples of a river strongly impacted by wastewater. For the first time, we demonstrated the co-occurrence of DTPMP and its transformation products glyphosate, AMPA, *N*-methyl-AMPA and IDMP in river sediment.

4 In-situ formation of glyphosate and AMPA in sewage sludge from phosphonates used as antiscalants and bleach stabilizers in households and industry

4.1 Abstract

The herbicide glyphosate and aminomethyl phosphonic acid (AMPA), a transformation product of glyphosate and other aminopolyphosphonates (APPs) are widespread pollutants in European rivers. We recently showed that besides rain-driven input after agricultural or urban herbicide application, municipal wastewater significantly contributes to glyphosate contamination in European rivers. The rather constant mass fluxes over the year made an explanation by herbicide applications difficult. In our search for a new source of glyphosate and AMPA, we here provide experimental evidence that a certain APP, used as antiscalant and bleach stabilizer in household detergents and numerous industrial processes, is a precursor of both glyphosate and AMPA. During incubation experiments with diethylenetriamine penta(methylene phosphonic acid) (DTPMP) in fresh activated sludge, we observed the formation of glyphosate with yields ranging from 0.017 to 0.040 mol% and formation of AMPA in the range of 0.402 to 1.72 mol% after 72 h. Both compounds are formed from DTPMP and possible intermediates, but they are also further transformed in consecutive reactions. Glyphosate formation from DTPMP was further proven by incubating ^{13}C-labeled DTPMP, which transformed into ^{13}C-glyphosate and ^{13}C-AMPA. The addition of DTPMP to azide-treated activated sludge yielded similar or even higher glyphosate and AMPA concentrations indicating that abiotic processes dominate the transformation process. The presence of MnO_x during incubation in biotic samples led to an increase in concentrations by factors of 2.3-3.1 for glyphosate and 1.8-2.6 for AMPA. In order to judge the relevance of this in-situ formation of glyphosate and AMPA from the laundry additive DTPMP, we estimated the average concentrations in wastewater.

4.2 Introduction

Amino(poly)phosphonates are high volume anthropogenic compounds used as powerful complexing agents in household products and industry. Currently, the commercially most important representatives of aminopolyphosphonates (APPs) are diethylenetriamine penta(methylene phosphonic acid) (DTPMP), aminotris(methylene phosphonic acid) (ATMP), and to a minor extent, ethylenediamine tetra(methylene phosphonic acid) (EDTMP) (for chemical structures see Fig. 16A). Important industrial applications of these compounds include cleaning processes, e.g. in the food industry, membrane treatment in reverse osmosis for drinking water production [26-29] and desalination,[30] prevention of scale and biofouling in cooling water,[31] as plasticizers in concrete[32] and other applications in the paper, metal and textile industries.[18] In household products,[25] APPs are used in laundry and cleaning products for bleach stabilization and antiscaling.

Reported European total quantities for phosphonate use ranged from 10,000-50,000 tons, of which 12,000 tons were used in household products (among them 4,094 tons DTPMP) in 2004[15] or 49,000 tons total phosphonate use in Europe in 2013[18] (10,000-12,000 in Germany).[192] A fraction of 25% was estimated to account for the German share.[193] The newest production numbers for Europe are available from REACH dossiers of the European Chemicals Agency, which lists the free acid of DTPMP and different salt forms (3 Na, 5-7 Na or NH_4), with the 5-7 sodium form having the largest estimated tonnage band of 10,000-100,000 tons per year.[194] For EDTMP, the calcium sodium salt has the largest tonnage band of 1,000-10,000 tons. More detailed numbers available for Germany are summarized in Table 6. Summarizing these data, we can estimate 1,500-2,500 tons of DTPMP (+EDTMP) use in Germany per year today. A different access to the amount of DTPMP used in Germany is possible from DTPMP concentrations in the influent of a wastewater treatment plant (WWTP) treating dominantly domestic wastewater. Combining the concentration data for the sorbed and the dissolved phase of 4-312 mg/kg (dry weight) and 7-71 µg/L and an average solid fraction of the influent of 300 mg/L,[78] total influent concentrations can be calculated to be between 19 and 165 µg/L. Extrapolated to 10 billion m^3 of wastewater in Germany,[195] loads of 200-1,650 tons of DTPMP entering WWTPs treating mainly domestic wastewater can be estimated (neglecting likely losses due to transformation during applications or in the sewer systems), which fits well to the numbers presented before.

We examined the formulations of German laundry products in 2023 available on the producers' websites. Many liquid detergents and several pods contained DTPMP, whereas powder detergents mostly contained 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP) also called etidronate. The use of liquid detergent increased in Germany in recent years.[193] Applications for domestic or industrial cleaning and laundry were assumed to have a wastewater relevance of almost 100%, other applications of 85%.[193] Retention of APPs during wastewater treatment is substantial with removal rates of 80-95% during phosphate elimination mainly via adsorption, but only 50% in case this treatment stage is missing.[16, 18]

Despite their intensive use, little is known about the environmental occurrence and fate of amino(poly)phosphonates.[18, 79] They are considered to be poorly biodegradable [18, 193] and have recently been detected at mg/kg concentrations in river sediments and sorbed to suspended particles.[22, 79] In sunlit waters, photolysis of magnesium-, calcium- or iron complexes is discussed to be a dominant transformation process.[196] In sediments, oxidation in the presence of Mn(II) and oxygen or manganese dioxide [197, 198] is a potential transformation pathway with aminomethyl phosphonic acid (AMPA), iminodi(methylene phosphonate) (IDMP) and phosphate as major transformation products.

The transformation processes of APPs in WWTPs remain largely uninvestigated; only the transformation products of ATMP have been described [38] but not of other APPs, mainly due to analytical constraints. Besides phosphate, the only known transformation product monitored intensively is AMPA,[26, 110] since it is also a major transformation product of the herbicide glyphosate. In our previous publication,[5] we conducted an extended analysis of temporal patterns of surface water concentrations of AMPA and glyphosate. Long concentration time series of both compounds showed a strong seasonality and a high correlation with wastewater markers such as pharmaceuticals but not with other herbicides suggesting wastewater to be a major source of glyphosate and AMPA. However, it is difficult to relate these seasonal patterns observed in Europe (but not in the USA) with (urban) herbicide applications. Instead, rather constants and in some rivers high mass fluxes of glyphosate and AMPA pointed to a different source. With the very similar patterns of AMPA and glyphosate and APPs as a known source of AMPA, we hypothesized that glyphosate may also be a transformation product of certain APPs.[5] As for the APP ATMP, its transformation in presence of MnO_x has already been demonstrated to form AMPA [121, 198] and the formation of glyphosate and AMPA from DTPMP was recently proven in aqueous solution in presence of MnO_2.[17] However, it is unclear, if this reaction would also process in environmental matrices such as activated sludge. Mn is found in high concentrations between 150-1,500 mg/kg Mn in primary and secondary sludge samples of two WWTP samples over two years [201] and 300 mg/kg Mn (dry mass) in activated sludge in WWTP Tübingen in 2023 (personal communication). Furthermore, deferrization sludge (DS) from drinking water containing high amounts of iron oxides and manganese is usually discarded via WWTPs and can further contribute to high concentrations inside WWTP.

Table 6: Compilation of data available for total phosphonate use or use of DTPMP (EDTMP) in Germany for different applications and years. For cleaning and laundry applications, the amounts of DTPMP were estimated from total sales to be 32%.[193] For other applications, the fraction is unknown.

Application	Tons/year	Data source	Ref.
washing and cleaning agents registered at the German Federal Office for Environment Protection	1,702 tons DTPMP 877 tons EDTMP	2001	[59]
domestic applications	6,000 tons total phosphonate of which 1,900 tons are DTPMP[a,b]	2024[a]	[25]
industrial laundry and dishwashing detergents	650-3,500 tons total phosphonates of which 208-1,120 tons are DTPMP[b]	2015	[199]
industrial laundry and dishwashing detergents	2,000 tons total phosphonates of which 640 tons are DTPMP[b]	2015	IHO[c], cited in [199]
industrial and domestic cleaning and laundry applications	4,900 tons in total of which 1,568 tons are DTPMP[b]	2008	[193]
applications other than cleaning and laundry (water treatment, cooling, paper-/textile industry, cosmetics, etc.)	4,900 tons total phosphonate	2008	[193]
antiscalants in cooling circles	1,500-2,200 total phosphonates but assumed to be mainly ATMP, HEDP, PBTC[200]	2002	[31]
all applications	9,800 tons total phosphonates of which 2,940 tons are DTPMP[b]	2013	[192]
antiscalant in drinking water osmosis	9 tons P from phosphonates released into surface waters	2019	[28]

[a] *sales numbers linearly extrapolated using data from 1994-2015 (not using later numbers due to a steep increase to 8,816 tons for 2017 being related to HEDP in dishwashing applications*

[b] *estimated assuming 32% of the total phosphonates to be DTPMP*[193]

[c] *Industrieverband Hygiene und Oberflächenschutz*

In this study, we investigate whether glyphosate forms from DTPMP in activated sludge. DTPMP was chosen due to its ethylene moiety relevant for glyphosate formation, its high production volumes and applications in household products. Using model calculations, we estimate whether this reaction can explain the high concentrations of glyphosate observed in European WWTP samples [13, 14, 87, 94, 119] and the rather constant mass fluxes throughout the year in rivers.[5] Furthermore, the influence of the addition of MnO_x on glyphosate and AMPA formation in activated sludge is investigated.

4.3 Materials and Methods

4.3.1 Chemicals

All chemicals were used without further purification.

Ultra-pure water (≥18 MΩcm^{-1}) was obtained using an ELGA-Veolia PURELAB Classic system (Celle, Germany) or a Milli-Q EQ 7000 pure water generator (MerckMillipore, Darmstadt, Germany) throughout the study.

Phosphonates: Diethylenetriamine penta(methylene phosphonic acid) standard was purchased from Zschimmer & Schwarz Chemie (Lahnstein, Germany) as free acid powder under the name "Cublen D 900 GR" with an active acid content of >90% (CAS: 15827-60-8). The purity was controlled by ^{1}H and ^{31}P-NMR.

Glyphosate (≥97%) from Toronto Research Chemicals (Toronto, Canada) was used for degradation experiments (see Section 4.3.3). Glyphosate-$^{13}C_3$-^{15}N (G172) (≥95%) from Cambridge Isotope Laboratories (Andover, USA) was spiked in incubation experiments in samples PS2 (see Table 7).

Chemicals for transformation reactions and extraction: Sodium azide (≥99.5%) for sludge incubations was obtained from Fluka (Buchs, Switzerland). From Sigma Aldrich (Steinheim, Germany) we bought sodium hydroxide (≥98%, pellets), sodium phosphate heptahydrate dibasic ($Na_2HPO_4 \cdot 7\ H_2O$) (≥99%) and Manganese (IV) oxide (MnO_2) (≥90%). Hydrochloric acid (37%, technical grade) for acidification of samples, sample extracts and synthesis purposes was bought from Brenntag (Reading, PA, USA).

Chemicals for analysis: Glyphosate and aminomethylphosphonic acid (AMPA) standards were purchased from the following companies: **CE-MS and LC-MS-1**: AMPA-^{13}C-^{15}N (AMPA112) from Toronto Research Chemicals (Toronto, Canada), AMPA-^{13}C-^{15}N-methylene-D_2 (≥95%) (AMPA114) from Cambridge Isotope Laboratories (Andover, USA), glyphosate-2-^{13}C (≥99.9%) (G169) and glyphosate-2-^{13}C-^{15}N (G170) (≥99%) from Sigma Aldrich (Steinheim, Germany). **LC-MS-2**: Glyphosate-^{13}C-^{15}N (G170) and AMPA-^{13}C-^{15}N-methylene-D_2 (AMPA114) were bought from Cambridge Isotope Laboratories (Andover, USA) and used as internal standards. Glyphosate and AMPA were obtained from Dr. Ehrenstorfer (Augsburg, Germany).

Chemicals for mass spectrometry were purchased from the following companies with LC-MS grade purity: **CE-MS and LC-MS-1**: Ammonium hydroxide solution (ca. 25%) from Sigma Aldrich (Steinheim, Germany), acetonitrile (ACN) from Honeywell (Seelze, Germany) and VWR Chemicals (Darmstadt, Germany), methanol from Th. Geyer (Renningen, Germany), formic acid, isopropanol and water from Merck (Darmstadt, Germany). **LC-MS-2**: Methanol (LC-MS grade) and ACN (LC-MS grade) from Carl Roth (Karlsruhe, Germany), ammonium carbonate from Sigma Aldrich (Steinheim, Germany). Aqueous sodium hydroxide (0.1 M) solution was obtained from Applichem Panreac ITW Companies (Darmstadt, Germany).

Chemicals for derivatization of DTPMP via methylation for **LC-MS-1** were bought from the following companies: trimethylsilyldiazomethane (1.2-2.4 M in hexane) from Thermo Fisher Scientific (Schwerte, Germany), aqueous sodium hydroxide (1 M) solution from Agilent Technologies (Santa Clara, CA, USA). Caffeine (anhydrous, ≥98.5%) from Carl Roth (Karlsruhe, Germany) was used as an internal standard to ensure stable ionization.

For the derivatization for **LC-MS-2**, fluorenylmethyloxycarbonyl chloride (FMOC-Cl) was purchased from Sigma Aldrich (Steinheim, Germany) and sodium tetraborate and ethylenediaminetetraacetic acid (EDTA) from Merck (Darmstadt, Germany).

Chemicals for synthesis and characterization of $^{13}C_5$-DTPMP: The synthesis of $^{13}C_5$-labeled DTPMP is described in the supporting information (see Section S1). For the synthesis of $^{13}C_5$-labeled DTPMP, we used ^{13}C-paraformaldehyde (>99 atom% ^{13}C), phosphorous acid (H_3PO_3, 99%) and diethylenetriamine (99%), all from Sigma Aldrich (Steinheim, Germany). Deuterium oxide (D_2O, 99.9 atom% D) (Sigma Aldrich, Steinheim, Germany) was used as a solvent for NMR analysis.

4.3.2 Samples and sample locations

Table 7 and Table 8 provide an overview of field samples from different WWTPs including sampling date and sample type.

WWTP Büsnau, Germany: The WWTP Büsnau consists of a primary sedimentation tank, denitrification tank, series of four aeration tanks and a secondary sedimentation tank. Additionally, the activated sludge is recycled between the secondary sedimentation step and the denitrification tank as well as between the last aerated chamber and the denitrification tank. The activated sludge has a retention time or “sludge age” of approximately 8 to 15 days. At the time of sampling, wastewater was received exclusively from the village of Büsnau (approximately 2,300 inhabitants) without any industrial input. Two weeks of dry weather preceded the sampling date, thus the amount of wastewater in the influent was low (dry weather inflow is commonly 2 L/s up to 8 L/s). Activated sludge samples for incubation experiments were collected on 2023-03-07, 11:00 AM from the first aeration tank using a plastic beaker and poured into a sterilized 1 L glass bottle. No rain was recorded at the station Renningen-Ihinger Hof for more than 7 days before the sampling (data: Deutscher Wetterdienst, DWD).

WWTP Pliezhausen, Germany: The wastewater is received exclusively from the village of Pliezhausen (inhabitant equivalent (IE) 4,500) with negligible industrial input. The WWTP is fed via a basin upstream of the sewage treatment plant, which feeds the WWTP, when a certain filling level is exceeded. The inflow to this WWTP varies seasonally, ranging from an average of 7 L/s (600 m^3/day) on dry days in midsummer to 12 L/s (1,000 m^3/day) in winter and 28 L/s (2,400 m^3/day) on rainy days. The WWTP Pliezhausen consists of a mechanical treatment stage with a rake and grit chamber, followed by a biological treatment in an aeration tank for both nitrification and denitrification, where oxygen is added using aeration rollers when the oxygen concentration is between 0.8 mg/L and 2 mg/L. Iron(III)chloride solution is continuously dosed at a rate of 2 L per hour for P-precipitation. Afterwards, the wastewater goes through secondary clarification where the sewage sludge is retained and the treated wastewater is discharged into the Reichenbach creek. In 2023, the sludge amounted to 1,900 m^3 (57 t dry weight), as reported by the plant operators. Activated sludge samples were collected from the aeration tank using a plastic beaker and poured into plastic bottles. For samples used for incubation experiments, special care was taken to maintain adequate oxygen levels in the activated sludge to prevent dieback prior or during incubation experiments. Activated sludge samples for incubation experiments were collected at different seasons on 2023-08-21, 8:30 AM (S1); 2023-09-27, 8:30 AM (S2), 2024-01-29, 9:00 AM (W1), 2024-03-18, 01:50 PM (W2), and 2024-06-10, 9:00 AM (S3).

In a preliminary campaign (PL1) at the WWTP Pliezhausen, various samples representative for the wastewater treatment process plus a sediment and water sample from the receiving water body Reichenbach were taken on 2023-06-26, 9:00 – 10:00 AM to be analyzed for glyphosate, AMPA and DTPMP.

Latest rain events before sampling recorded at the station Unterjesingen (DWD) within 5 days before sampling: PS1 (2023-08-21): light rain on 2023-08-16: 2.7 mm (4-6 PM), 0,1 mm (7-8 PM), 2023-08-17: 0.1 mm (5-6 AM). PS2 (2023-09-27): 2023-09-22: 1.8 mm (12-2 AM), 0.2 mm (4-5 AM), 0.1 mm (7-8 PM). PW1 (2024-01-29): 2024-01-24: 4.9 mm (2-4 PM), 2024-01-26: 0.5 mm (3-5 PM), 2024-01-27: 0.1 mm (10-11 AM), 2024-01-28: 0.1 mm (09-10 AM). PW2 (2024-03-18): 2024-03-13: 15.1 mm (12 AM – 2 PM), 2024-03-15: 1.5 mm (9-12 PM), 2024-03-16: 0.4 mm (5-9 AM). PS3 (2024-06-10): 2024-10-04 to 2024-10-06 showing six hours of light rain ≤0.2 mm each on total.

<u>WWTP Tübingen, Germany:</u> The relatively large municipal WWTP in Tübingen uses mechanical rakes, a grit chamber, biological secondary treatment, chemical flocculation and precipitation and ozonation for quaternary treatment. A sample of activated sludge was taken on 2024-06-17 to be used in a separate incubation experiment (Tü-OECD) where we followed the OECD Guideline 302B on inherent biodegradability.

<u>Sludge from drinking water deferrization:</u> Sludge samples from deferrization of drinking water, were kindly provided by the Berliner Wasserbetriebe, Germany. This sludge is transferred to WWTPs for waste disposal. The sludge contains elevated amounts of manganese. Using MQuant manganese test stripes from Merck, Darmstadt, Germany, we detected a manganese concentration of 20 mg/L in the supernatant. Analysis of the sludge was not possible due to the intense color from iron oxide. With pH indicator stripes we measured a pH of 7. The deferrization sludge (DS) was stored at 6 °C.

4.3.3 Protocols for DTPMP transformation experiments and sample processing

During WWTP sampling campaigns, samples for incubation experiments were processed immediately (within 1 h) when arriving in the laboratory. Samples from incubation experiments were either analyzed directly or immediately frozen and freeze-dried afterwards. Various samples from the WWTP Pliezhausen and the receiving river Reichenbach were processed directly after sampling or after freeze-drying to be analyzed for glyphosate, AMPA and DTPMP (samples PL1) in the raw samples. In the following we refer to the activated sludge suspension taken directly from the WWTP aeration tank as “raw activated sludge”. The sludge samples used for incubation experiments were obtained after sedimentation and decantation of aqueous supernatant and are referred to as “sludge”. We used two different ways of processing the samples prior to analysis: The samples were either centrifuged and then extracted or freeze-dried prior to extraction. Results for freeze-dried samples are presented in the main text, while those for centrifuged samples are included in the supporting information together with the error bars from three parallel experiments.

4.3.3.1 Incubation of DTPMP or glyphosate in fresh activated sludge

A total of six sets of incubation experiments were carried out. DTPMP (as a commercial standard, $^{13}C_5$-DTPMP synthesized in-house or a commercial product of DTPMP) or labeled glyphosate were spiked for incubation in fresh activated sludge and all samples were analyzed for glyphosate and AMPA. Details of the incubation experiments are summarized in Table 7. The proportion of sludge in the samples varied between the sampling campaigns in Pliezhausen. In April 2024, the dry matter content (freeze-dried) of activated sludge was 0.75% whereas in June 2024, it was 0.98%. Upon transfer to the laboratory, incubation experiments with DTPMP or glyphosate were prepared immediately and started approximately one hour after sampling. For the incubation experiments, either

25 g (± 1.3%) (Büs- and PS1-samples) or 35 g (± 0.6%) (P-samples except PS1) of (fresh activated) sludge were used per experiment.

The basic protocol was as follows: Blank or spiked samples in 50 mL polypropylene tubes (Cellstar tubes, Greiner Bio-One, Frickenhausen, Germany) were placed on a shaker (125 rpm) at ambient temperature. Blank controls (biotic conditions, BC) were not treated further. For abiotic controls (abiotic conditions, AC), an aqueous solution of sodium azide (10%) was added to reach a final sludge concentration of 1% sodium azide. For incubation, aqueous solutions of DTPMP (10 g/L), isotope-labeled $^{13}C_5$-DTPMP (20 g/L synthesis mixture) (see Section S2-1 and Table S2-1) or isotope-labeled glyphosate-$^{13}C_3$-^{15}N (G172) (700 mg/L) were added to reach the final concentrations listed in Table 7. At the time points indicated in Table 7, an aliquot of 2.5 mL of the suspension was taken and freeze-dried. The samples were extracted according to Wimmer et al.[73] (protocol described in Section 4.3.4.1) and analyzed for glyphosate and AMPA using CE-MS (for analytical details, see Section 4.3.4, CE-MS). For experiment PS2-G172, for unknown reasons, we observed run failures with the extracts of the freeze-dried samples even when repeating the extraction. For this experiment, we thus show the results for centrifuged samples.

Table 7: Incubation experiments with DTPMP, $^{13}C_5$-labeled DTPMP and $^{13}C_3$-^{15}N-labeled glyphosate (G172) in inocula of (fresh activated) sludge, BC = biotic conditions AC = abiotic conditions after addition of 1% sodium azide, DS = addition of deferrization sludge from drinking water deferrization). For the incubation in a sample from the WWTP Tübingen (Tü-OECD), a commercial sample of DTPMP was used. Spiking concentrations were calculated for sludge. For the incubation experiments, either 25 g (Büs- and PS1-samples) or 35 g (P-samples except PS1) of fresh activated sludge (after sedimentation and decantation) were used per incubation, which was carried out in triplicate for each type of experiment. For the samples S3, DTPMP was spiked over a range of 50-500 mg/kg in duplicates.

Experimental setup			**Spiked substance (final concentration in sludge)**	**Sampling times (h)**
Sample from Büsnau				
Büs screening (2023-03-07)	-BC		blank	0, 24, 48, 72
	-AC		1% sodium azide	
	-DTPMP	-BC	625 mg/kg (± 2.9%) DTPMP	
Samples from Pliezhausen				
PS1 Screening (2023-08-21)	-BC		blank	0, 24, 48, 72, 96
	-AC		1% sodium azide	
	-DTPMP	-0.5-BC	497 mg/kg (± 1.2%) DTPMP	
		-1.0-BC	978 mg/kg (± 1.7%) DTPMP	
PS2 glyphosate transformation (2023-09-27)	-G172	-BC	9.97 mg/kg (± 0.3%) G172	0, 2, 4, 6, 22, 29, 49
		-AC	10.06 mg/kg (± 0.9%) G172 + 1% sodium azide	
PW1 relevance of abiotic reactions (2024-01-29)	-BC		blank	0, 3, 6, 24, 27, 48, 72
	-AC		1% sodium azide	
	-DTPMP	-0.75-BC	738 mg/kg (± 0.9%) DTPMP	
		-0.75-AC	742 mg/kg (± 0.5%) DTPMP + 1% sodium azide	
		-0.75-DS	738 mg/kg (± 0.2%) DTPMP + 1 mL DS	
		-0.75-DS-AC	735 mg/kg (± 0.5%) DTPMP + 1 mL DS + 1% sodium-azide	
		-0.75-MnO_2	737 mg/kg (± 0.9%) DTPMP + 5.71 mg/kg MnO_2	
		-0.75-MnO_2-AC	737 mg/kg (± 0.6%) DTPMP + 5.71 mg/kg MnO_2 + 1% sodium-azide	
PW2 formation vs. sorption and quant. assessment (2024-03-18)	-BC		blank	0, 17, 25, 48, 72, 96, 168
	-AC		1% sodium azide	
	-13C-DTPMP	-0.6-BC	596 mg/kg (± 0.8%) $^{13}C_5$-DTPMP*	
		-0.6-AC	596 mg/kg (± 0.5%) $^{13}C_5$-DTPMP* + 1% sodium azide	
PS3 concentration series for DTPMP (2024-06-10)	-BC		blank	0, 48, 72, 96, 168
	-AC		1% sodium-azide	
	-DTPMP	-0.05-BC	51 mg/kg (± 0.7%) DTPMP	
		-0.05-AC	52 mg/kg (± 0.2%) DTPMP + 1% sodium azide	
		-0.10-BC	100 mg/kg (± 0.4%) DTPMP	

Experimental setup			Spiked substance (final concentration in sludge)	Sampling times (h)
PS3 concentration series for DTPMP (2024-06-10)	-DTPMP	-0.10-AC	100 mg/kg (± 0.1%) DTPMP + 1% sodium azide	0, 48, 72, 96, 168
		-0.25-BC	251 mg/kg (± 0.02%) DTPMP	
		-0.25-AC	252 mg/kg (± 0.3%) DTPMP + 1% sodium azide	
		-0.50-BC	498 mg/kg (± 0.5%) DTPMP	
		-0.50-AC	500 mg/kg (± 0.04%) DTPMP + 1% sodium azide	
Sample from WWTP Tübingen for OECD Test 302B				
Tü OECD test (2024-06-17)	-OECD		5000 mg/kg commercial product of DTPMP	0, 3, 48, 96, 168, 216, 408

** Calculated assuming a content of 40.5 wt% $^{13}C_5$-DTPMP in the synthesis mixture (for synthesis and composition, see Section S2-1 and Table S2-1)*

4.3.3.2 OECD test 302B for inherent biodegradability

In a separate incubation experiment, we followed the OECD Guideline 302B [24] on inherent biodegradability (Tü-OECD) in cooperation with CHT Germany GmbH, Tübingen. Activated sludge was sampled from the large WWTP in Tübingen on 2024-06-17. A commercial sample of DTPMP synthesized similarly as described in Section S2-1 was used and added as an aqueous solution (1 g/L commercial DTPMP) to a final concentration of 50 mg/L dissolved organic carbon. This equals 5000 mg/kg commercial DTPMP (assuming 100% purity). Inocula were prepared using 4 L of activated sludge obtained after sedimentation and removal of the supernatant. The sludge suspension was washed twice with mineral medium according to OECD 302B to reach a final dissolved organic carbon (DOC) <1 mg/L and a particle concentration of 0.2 g/L (dry weight, that is 0.02% assuming a density of 1 mg/g). 500 mL of this suspension were mixed with mineral medium, water and aqueous NaOH (for pH control) to 5 L. Two blank samples were run in parallel (duplicates each). To be able to sample at different time points, CO_2 evolution was not followed. We sampled 250 mL aliquots of the suspension after homogenization at the time points chosen: directly after the addition of DTPMP, after 3 h and then at intervals of several days (see Section 4.3.3 for details). The last sample was taken after 408 h (17 days). The samples were processed immediately and split into four subsamples using four 50 mL polypropylene tubes and then centrifuged at 1,520 g. After removal of the supernatant the pellet was frozen at -80 °C until freeze-drying and then extracted and measured as described in Section 4.3.4.

4.3.4 Analytical methods

4.3.4.1 Extraction of glyphosate, AMPA and DTPMP from sediments and sludge

The sediment sample was freeze-dried and sieved to a particle size <63 µm. Sediment and activated sludge samples were extracted according to Wimmer et al.[70] Aliquots of raw activated sludge or aliquots of sludge taken during the incubation experiments were sampled. We extracted 200 mg (± 1%) of freeze-dried sediment or 14 mg (± 1%) of freeze-dried sludge sample. Due to the small amount of sample available after freeze-drying, samples from triplicate incubations were pooled. Section S2-3 describes the sample preparation for the analysis of centrifuged sludge samples in triplicate.

The extraction of glyphosate, AMPA and DTPMP was conducted as follows: A liquid:solid ratio of 2.5 was chosen for the sediment sample. For freeze-dried sludge samples, the liquid:solid ratio was 12.5. The extraction medium was an aqueous solution of a mixture of 50 mM NaOH and 50 mM Na_2HPO_4 with a concentration of 400 µg/L of the isotope-labeled internal standards G170 and AMPA112 or AMPA114. In preliminary experiments with sludge samples from the WWTP Büsnau and samples from the first sampling campaign (PL1) (see Section 4.4.2) internal standards were added to the sample after extraction and thus prior to injection for CE-MS. Sample extracts were stored at -20 °C and measured as soon as possible but not later than two days. In our study, extraction efficiencies for glyphosate and AMPA from freeze-dried samples were high with 119% for glyphosate and 103% for AMPA on average. The extraction efficiencies for DTPMP could not be quantified with the current method.

4.3.4.2 Separation methods

In this study, we mainly used capillary electrophoresis-mass spectrometry (CE-MS) to determine glyphosate and AMPA concentrations and LC-MS for DTPMP analysis after derivatization with trimethylsilyldiazomethane (LC-MS-1). Glyphosate formation was further confirmed using LC-MS after derivatization with FMOC (LC-MS-2).

Capillary electrophoresis–mass spectrometry (CE-MS): CE-MS analysis used a 7100 Agilent CE System (Agilent Technologies, Waldbronn, Germany) coupled to the Agilent 6550 iFunnel QToF-MS instrument (Agilent, Santa Clara, CA) also used for LC-MS-1 analysis. We used the CE-MS method published for glyphosate analysis by Wimmer et al.[70] with adaptations indicated in italics: *bare fused silica capillaries* with an inner diameter of 50 µm from Polymicro Technologies (Phoenix, USA) were used with a length of *75 cm or 100 cm*. Before use, the outer coating was removed at the outlet of the capillaries using a lighter and the capillary tip was sanded to a 90 ° angle to ensure good conditions at the MS interface. Measurements were conducted at 25 °C. The capillary was flushed with BGE for 170 s prior to each measurement. The sample injection was carried out by applying a pressure of *100 mbar for 15 s, 75 mbar for 15 s or 75 mbar for 30 s depending on matrix effects*. After sample injection, a plug of background electrolyte (BGE) was injected (*100 mbar pressure for 10 s*) to prevent diffusional losses into the BGE vial. An additional inlet pressure of *50 mbar (for 100 cm capillaries) or 40 mbar (for 75 cm capillaries)* was applied during the CE-MS analysis to reduce analyte migration times and provide stable electrospray conditions. *After each measurement, the capillary was flushed with aqueous 50 mM NaOH solution for 25 s and flushed with BGE for 80 s after dipping the electrode into a vial with water to avoid carryover effects. The electrospray voltage was set to -3.5 kV.* The fragmentor voltage was at 175 V for the first 12 s of the measurement and then set to 380 V*. The octopole voltage was set to 750 V.* A 1:1 (v/v) mixture of isopropanol and ultra-pure water containing 0.01% formic acid at a flow rate of 5 µL/min served as the sheath liquid.

Liquid chromatography-mass spectrometry 1 (LC-MS-1) with trimethylsilyldiazomethane derivatization: For LC-MS analysis of DTPMP, we followed the derivatization protocol of Wang et al.[65] using trimethylsilyldiazomethane as derivatization agent. Aqueous 1 M NaOH solution was added to neutralize aqueous samples from WWTPs and receiving river prior to derivatization and after ion exchange using Dionex OnGuard II H cartridges (1 cc, Thermo Fisher Scientific, USA). This is a strongly acidic polystyrene cation-exchanger in the H^+ form. LC-MS analysis was conducted with an Agilent 1260 Infinity LC system (Agilent, Waldbronn, Germany) coupled to the Agilent 6550 iFunnel QToF-MS instrument, which was also used for CE-MS analysis. Differences to the MS parameters used for CE-MS were: For ionization, a Dual

Agilent Jet Stream ion source was used in positive ionization mode. The nebulizer pressure was set to 35 psi, the drying gas temperature to 160 °C, the drying gas flow rate to 16 L/min and the fragmentor voltage to 360 V. The sheath gas was supplied at a flow rate of 11 L/min at 325 °C. The mass range was 80-1200 m/z. Internal calibration was carried out using purine (121.0503 m/z) and HP-921 (922.0098 m/z) (both from Agilent Technologies, Waldbronn, Germany) introduced via a reference sprayer. Analytes were separated on an InfinityLab ZORBAX Eclipse Plus C18 column (2.1 x 150 mm, 3.5 µm) connected to a Zorbax Eclipse Plus C18 guard column (2.1 x 12.5 mm, 5 µm, Agilent Technologies).

The mobile phases used were: Eluent A (water + 0.1% formic acid) and Eluent B (ACN + 0.1% formic acid). The following mobile phase gradient was used: hold 5% B for 1 min, linear ramp to 95% B over 7 min, hold 95% B for 7 min, then return to initial conditions within 1.5 min, and equilibrate at initial conditions for additional 6.5 min prior to the next injection. The column temperature was maintained at 25 °C, and the mobile phase flow rate was 0.3 mL/min. A sample volume of 2 µL was injected for LC-MS analysis. The injection needle was dipped into a 1:1 mixture of ACN and water (both LC-MS grade) before injecting the sample into the sample loop to prevent carryover.

Liquid chromatography-mass spectrometry 2 (LC-MS-2) with FMOC derivatization: In order to verify analytical results, selected samples were subjected for confirmative analysis by LC-MS analysis after FMOC derivatization, following DIN ISO 16308:2017-09. For the derivatization of glyphosate and AMPA, a protocol of the Zweckverband Landeswasserversorgung Langenau was followed. Initially, a 1 mL aliquot of the sample was mixed with 25 µL of the isotope-labeled internal standard ^{13}C-^{15}N-glyphosate (G170) (10 µg/L, in MeOH) and ^{13}C-^{15}N-D_2-AMPA (AMPA114) (40 µg/L, in MeOH) and 25 µL of EDTA (0.1 mol/L) in a glass vial. Subsequently, 120 µL of aqueous borate buffer (5% di-sodium tetraborate decahydrate, pH 9) and 120 µL of an FMOC-Cl solution (12 mg/L in ACN) were added. Samples were homogenized and the reaction was allowed to proceed overnight in the dark. After derivatization, samples were filtered using a syringe filter (RC, 0.20 µm, 15 mm from Macherey-Nagel, Düren, Germany).

FMOC-derivatized samples were analyzed using a Shimadzu LC 30 liquid chromatography system (Shimadzu Duisburg, Germany) coupled to a Sciex QTrap 5500 mass spectrometer (SCIEX, Framingham, USA). The separation was performed on a reversed-phase Kinetex 2.6 µm EVO C18 100 Å column. A gradient elution was performed with Eluent A consisting of MilliQ water with 2 mM ammonium carbonate, and Eluent B consisting of methanol with 2 mM ammonium carbonate. The mobile phase gradient was programmed as follows: starting with 20% B, the proportion of Eluent B was increased to 50% B in 6 min. This was followed by a linear ramp to 90% B in 1 min. This composition was held constant for 3 min. The gradient then returned to initial conditions in 0.1 min with a total run time of 15 min. The column temperature was maintained at 25 °C, and the mobile phase flow rate was 0.35 mL/min. The injection volume of samples was 50 µL. The mass spectrometer source parameters were set as follows: nebulizer gas at 60 psi, heater gas at 50 psi, entrance potential at 10 V, collision gas at 940 psi, ion spray voltage -4.5 kV and temperature 600 °C. For identification and quantification, the following mass-transitions were monitored: $390.1 \rightarrow 168.0$ for glyphosate-FMOC, $392.0 \rightarrow 170.0$ for glyphosate-FMOC-^{13}C-^{15}N, $332.0 \rightarrow 110.0$ for AMPA-FMOC and $336.0 \rightarrow 114.0$ for AMPA-FMOC-^{13}C-^{15}N-D_2.

Details on **NMR analysis** are provided in the supporting information in Section S2-2.

4.4 Results and Discussion

In this study, we first present a potential reaction scheme for the transformation reaction of DTPMP to glyphosate and AMPA based on comparable pathways known for glyphosate transformation. In the second part, we investigate the co-occurrence of DTPMP, glyphosate and AMPA in WWTP samples. Third, in controlled laboratory experiments, we study the formation of glyphosate and AMPA from DTPMP during incubation of DTPMP in activated sludge under biotic and abiotic conditions after azide treatment.

4.4.1 Proposed transformation pathway from DTPMP to glyphosate and AMPA

In Fig. 16, we introduce a possible transformation pathway based on known bond cleavage reactions for glyphosate. We start with the well-known transformation of glyphosate to AMPA in Fig. 16B,[202] which proceeds via the cleavage of the C-N bond near the carboxylic acid moiety. The resulting amine is AMPA. On the carbon site, the aldehyde glyoxylic acid is formed.

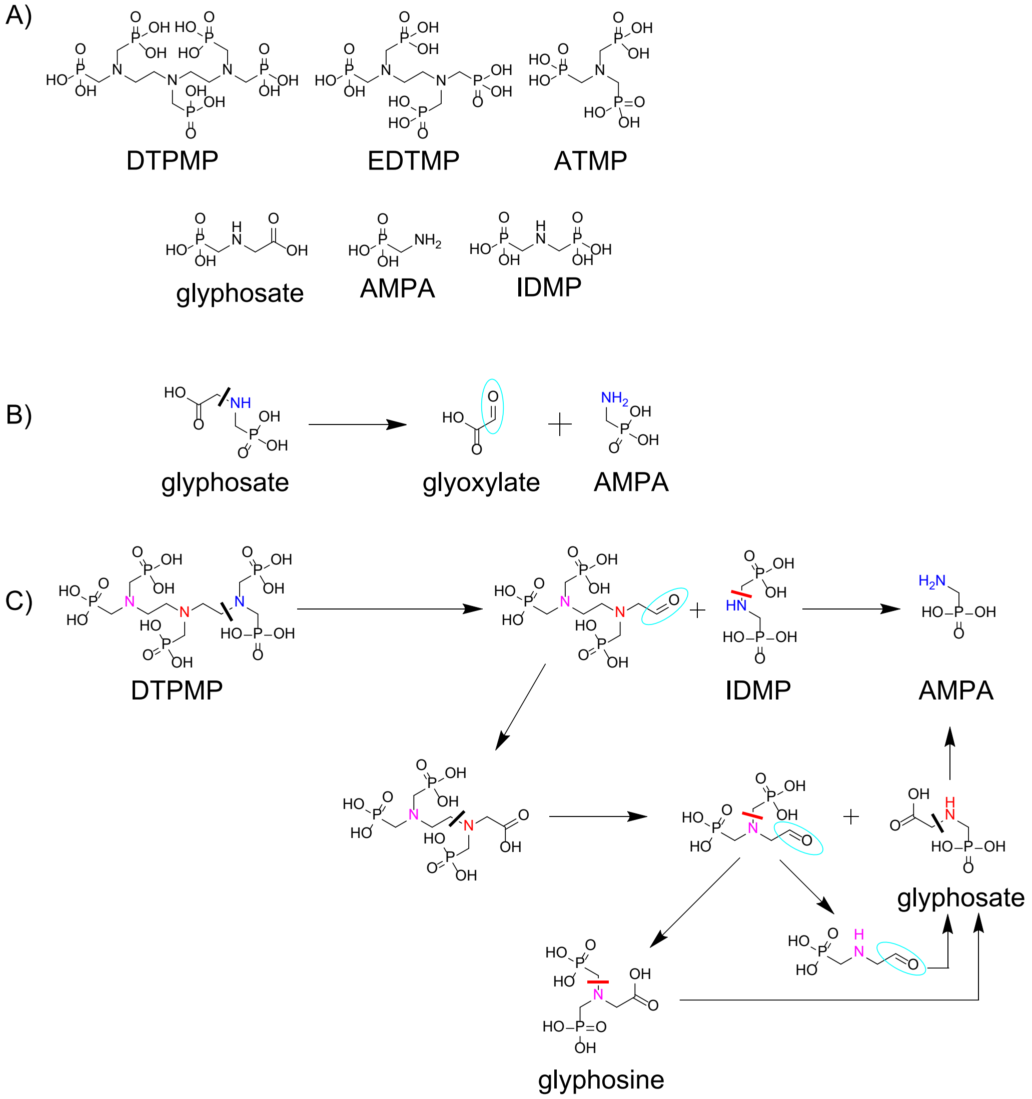


Fig. 16: (A) Structures of the commercially most important aminopolyphosphonates (free acids): DTPMP: diethylenetriamine penta(methylene phosphonic acid), EDTMP: ethylenediamine tetra(methylene phosphonic acid), ATMP: aminotris(methylene phosphonic acid) and glyphosate as well as IDMP: iminodi(methylene phosphonate) and AMPA: aminomethyl phosphonic acid. (B) Reaction scheme for the formation of AMPA and the aldehyde glyoxylate from glyphosate.[202] (C) Proposed simplified reaction scheme for the formation of glyphosate and AMPA from DTPMP.

Next, we transfer this reaction step to the ethylene diamine unit in DTPMP. Exemplarily, a C-N bond near an outer nitrogen is broken (see Fig. 16C). The amine evolving is IDMP, often described as a transformation product of APPs like DTPMP, e.g. in mixed abiotic-biotic processes in cell culture media containing manganese salts.[181, 203] With the central ethylene diamine chain, the aldehyde forming is an acetaldehyde. This aldehyde can be expected to be further oxidized to the acetic acid derivative (now or possibly at a later stage of the reaction). If a further C-N bond is cleaved, the amine formed is glyphosate. The other product of this cleavage is another acetaldehyde, which can either form glyphosine upon oxidation, known to be converted to glyphosate in microbially mediated reactions [181] or another small acetaldehyde, which may be oxidized to a second glyphosate molecule. The scheme shows that AMPA may form in a consecutive reaction from glyphosate but also via other pathways.

Surely, other similar transformation pathways can be proposed, a detailed and more mechanistic study is part of our current research. Recently, the formation of glyphosate from DTPMP was demonstrated in the presence of MnO_2 in aqueous solution.[17] Similarly, glyphosate formation from EDTMP, containing the same central ethylenediamine unit as DTPMP, was reported during ozonation [125, 204] with a similar mechanism proposed [125] and UVC irradiation.[125] In principle, the reaction schemes proposed in Fig. 16 may account for abiotic and biotic transformation as both processes are discussed to involve radical reactions,[205, 206] leading to similar transformation products as described in the studies cited in this paragraph.

4.4.2 Simultaneous occurrence of DTPMP, AMPA and glyphosate in samples from wastewater treatment

So far, DTPMP was only rarely analyzed in WWTP samples with the most recent study stating concentrations of 100-250 mg/kg of freeze-dried adsorbed suspended matter and 7-71 µg/L dissolved DTPMP in WWTP influent [78] and in river sediments upstream (6.7–29.4 mg/kg) and downstream (17.8–53.5 mg/kg) of WWTPs.[22] However, DTPMP was not yet determined in sewage sludge. Sorption to sewage sludge is discussed to be the main elimination route for DTPMP in a WWTP.[18] We analyzed samples from a WWTP (influent, effluent, activated sludge) and in sediment samples of the receiving water. In the same samples, we also analyzed glyphosate and AMPA as suspected vs. known transformation products of DTPMP. For sludge samples, this provided information on background concentrations. DTPMP was detected in all samples of sampling campaign PL1 as shown in Table 8: in WWTP influent, in the influent and effluent of the second purification step, in activated sludge, in the final effluent and, finally, in the river water. However, we could not quantify DTPMP concentrations due to matrix effects. Glyphosate was detected at elevated concentrations of up to almost 3 mg/kg in suspended matter of the influent and in sludge (LOQ = 0.8 mg/kg in freeze-dried sludge). AMPA signals had a signal-to-noise ratio >3, but we failed to quantify AMPA due to strong matrix effects and instrumental constraints. A secondary extraction was not made as ongoing transformation reactions would falsify the results.

The presence of such high concentrations of glyphosate from herbicide applications would be unexpected as the PL1 samples were all taken during dry weather conditions (for details see Section 4.3.2). These conditions make glyphosate and AMPA mobilization after urban applications unlikely as discussed by Märki.[119] High concentrations of glyphosate (0.5-3 mg/kg) and AMPA (2-21 mg/kg) were also reported over one year by Ghanem et al.[87] in dried sewage sludge from the WWTP Elancourt, mainly treating domestic wastewater, who asked for more research on the role of APPs for the presence glyphosate and AMPA.

Table 8: Glyphosate and AMPA concentrations in various samples from the WWTP Pliezhausen (first sampling campaign PL1), including sediments from the receiving water body Reichenbach. DTPMP could not be quantified with sufficient precision in the samples using the current analytical methods, so we only indicate whether it was detected or not. Glyphosate concentration data are reported for freeze-dried samples, see Section 4.3.4.1 for sample preparation. AMPA samples could not be quantified due to an insufficient concentration of the internal standard. Glyphosate and AMPA were analyzed by CE-MS and DTPMP by LC-MS-1 (see Section 4.3.4).

samples from PL1 and Reichenbach (2023-06-26, 9:00 – 10 AM)	**glyphosate**	**AMPA**	**DTPMP**
PL1 – influent after mechanical treatment	Not measured	Not measured	Detected
PL1 – influent second clarification	2.9 mg/kg in freeze-dried suspended matter	Detected in freeze-dried suspended matter	Detected in the supernatant
PL1 – effluent second clarification	Not measured	Not measured	Detected in the supernatant
PL1 - sludge	2.4 mg/kg in freeze-dried sludge	Detected in freeze-dried sludge	Detected in the supernatant
Reichenbach	32 µg/kg in freeze-dried sediment (≤63 µm)	Detected in freeze-dried sediment	Detected in river water

4.4.3 Formation of glyphosate during the transformation of DTPMP in activated sludge

In order to test our hypothesis that DTPMP might be a precursor for glyphosate in activated sludge, we conducted different sets of experiments incubating fresh activated sludge with 50 to 1000 mg/kg pure commercial DTPMP standard (equaling 6-121 g/kg dry weight (freeze-dried samples)), see Table 7. First, two screening tests were made using activated sludge from two different WWTPs in Büsnau and Pliezhausen (samples Büs and PS1). Second, we investigated the relevance of abiotic transformation including azide-treated samples for both DTPMP and glyphosate as spiked compounds (commercial standards) (samples PW1 and PS2). Third, we used $^{13}C_5$-labeled DTPMP synthesized in-house, which is not commercially available, to discriminate formation from possible (de)sorption. Finally, the dependence of glyphosate formation on the DTPMP starting concentration was evaluated. The investigated WWTPs dominantly treated domestic wastewater at the time of sampling.

4.4.3.1 In-situ formation of glyphosate from DTPMP in activated sludge

First screening experiments used activated sludge from the WWTPs in Büsnau and Pliezhausen, sampled after extended dry weather periods (for details, see Section 4.3.2) and with DTPMP concentrations between 500 and 1000 mg/kg sludge. For the samples in Büsnau, we observed a strong decrease in the peak area of DTPMP over 72 h of incubation (see Fig. S6). After 72 h of incubation, glyphosate concentrations in freeze-dried samples increased from <LOQ (0.3 mg/kg) to about 11 mg/kg glyphosate for PS1-DTPMP-0.5-BC and similarly to 8 mg/kg glyphosate for PS1-DTPMP-1.0-BC. AMPA concentrations in freeze-dried sludge reached 85 and 111 mg/kg in the two treatments after 72 h (Fig. 17A and B) with an LOQ of 0.63 mg/kg for AMPA in freeze-dried sludge. Then, concentrations slightly diminished. We presume that AMPA reached its concentration maximum in the consecutive reaction and its further transformation starts to dominate the reaction. Concentrations of glyphosate in biotic and abiotic controls without DTPMP added remained close or below the LOQ of 0.3 mg/kg glyphosate in freeze-dried sludge.

With these first strong hints for an in-situ formation of glyphosate in activated sludge, the experiments were expanded with fresh activated sludge taken in Pliezhausen in winter (samples PW1). Glyphosate concentrations increased from <LOQ to 5 mg/kg in freeze-dried samples after 72 h of incubation, similar to the PS1 samplings, see Fig. 17C and Table 9. AMPA concentrations in winter samples (PW1-DTPMP-0.75-BC) were lower in the first 24 h of the experiment than in summer (PS1-DTPMP-BC), but increased to a similar maximum concentration of 99 mg/kg after 48 h, see Fig. 17D and Table 9. Finally, AMPA concentrations decreased to 79 mg/kg after 72 h. In the biotic and abiotic controls without DTPMP (PW1-BC and PW1-AC), a slight increase of AMPA concentrations was observed, but they remained close to the LOD. We attribute this to a formation from residual DTPMP (or some glyphosate) already present in the fresh activated sludge samples. Differences in summer and winter samples can be expected from differences in temperature and seasonal microbial activities,[207, 208] likely relevant at least in the transformation of smaller (amino)phosphonate transformation products such as glyphosate.

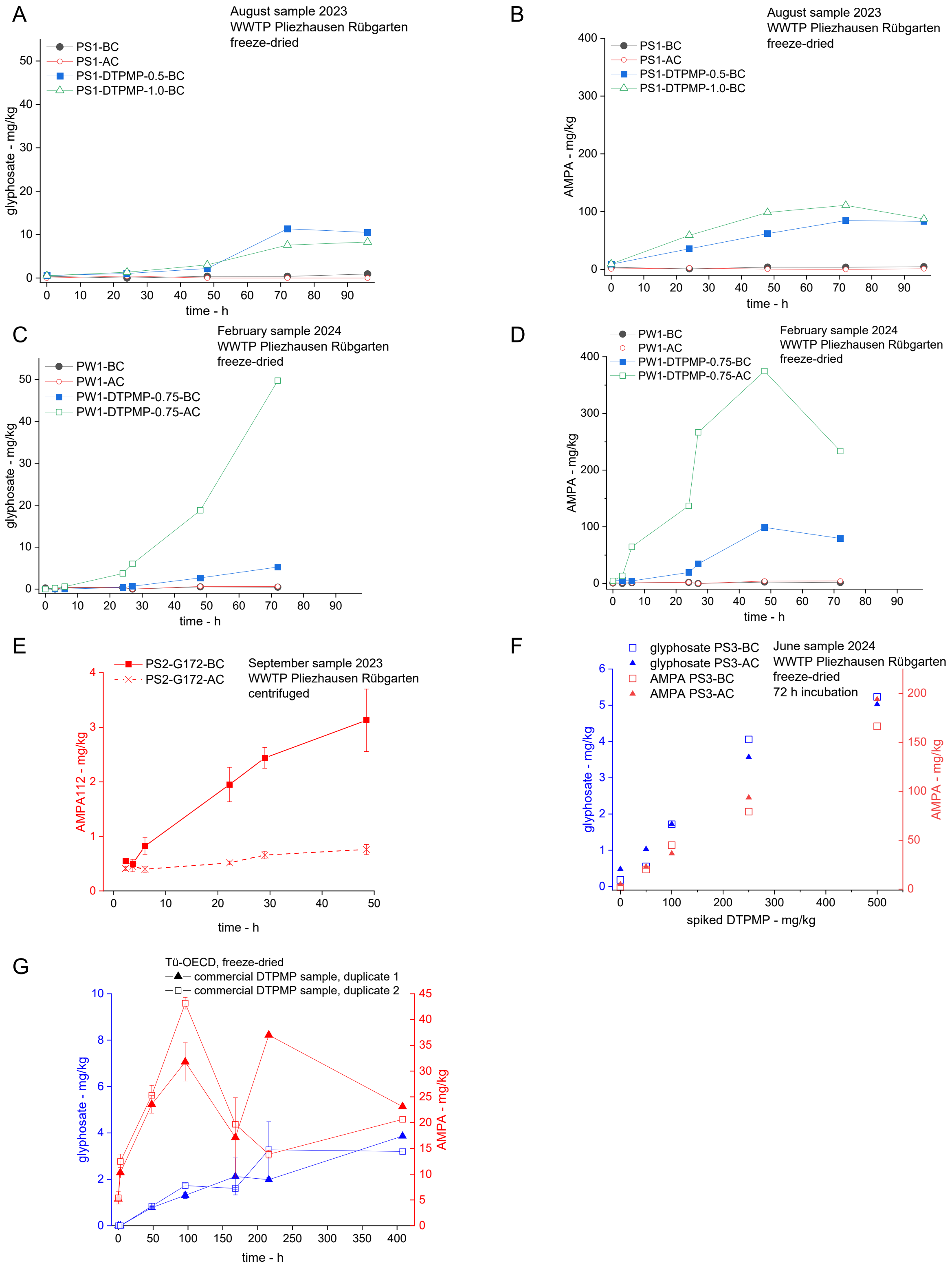


Fig. 17: Glyphosate and AMPA concentration from incubation experiments in activated sludge taken from (A-F) WWTP Pliezhausen and (G) from WWTP Tübingen. (A-D) Concentrations in freeze-dried sludge after spiking sludge with 500-1000 mg/kg DTPMP in different sampling campaigns as indicated in the names of the experiments provided in the panels (sampling PS1 and PW1, details in Table 7). (E) AMPA112 concentrations in centrifuged sludge during incubation of 10 mg/kg G172 (sampling PS2). (F) Concentration data after 72 h of incubation of 50-500 mg/kg DTPMP shown as a function of the spiking concentration (sampling PS3). (G) Following the OECD 302B test guideline (see Section 4.3.3.2), a commercial sample of DTPMP was spiked to fresh and washed activated sludge from the WWTP Tübingen and incubated for 408 h (Tü-OECD) (Details in Table 7). Results of blank samples were <LOD. Experimental details in Section 4.3.3, Section S2-3, and Table 7. Analysis by CE-MS (Section 4.3.4). Concentrations after 72 h are compiled in Table 9.

Table 9: Concentrations and concentration ratios for glyphosate and AMPA. Samples taken in Pliezhausen, PW1 and PS3. Results for 72 h of incubation with DTPMP. Analytical results for pooled freeze-dried samples from triplicate incubations and quantification by CE-MS. Concentration ratios determined for treatments with/without azide (AC/BC). AC = abiotic conditions; BC = biotic conditions. Sample names as defined in Table 7. Plots for the concentration changes over time of PW1 and PS3 are presented in Fig. 17C-D and F, respectively.

Sample name			**Glyphosate (mg/kg)**	**AMPA (mg/kg)**	**Ratio AC/BC glyphosate**	**Ratio AC/BC AMPA**
PW1	-BC		0.4	1.6	1.4	2.8
	-AC		0.6	4.4		
	-DTPMP	-0.75-BC	5.3	79.5	9.5	2.9
		-0.75-AC	49.7	233.5		
PS3	-BC		0.2	1.7	2.5	2.8
	-AC		0.5	4.8		
	-DTPMP	-0.05-BC	0.6	20.0	1.7	1.1
		-0.05-AC	1.0	22.8		
		-0.10-BC	1.7	44.9	1.0	0.8
		-0.10-AC	1.7	36.3		
		-0.25-BC	4.1	79.2	0.9	1.2
		-0.25-AC	3.6	93.5		
		-0.50-BC	5.2	166.4	1.0	1.2
		-0.50-AC	5.0	193.9		

To better understand the dependence of glyphosate and AMPA concentration on the starting concentration of DTPMP, we prepared a DTPMP concentration series of 50-500 mg/kg in sludge (sampling campaign PS3 in June 2024). As shown in Fig. 17F, glyphosate and AMPA concentrations almost linearly depended on the amount of DTPMP spiked.

DTPMP is regarded persistent, which is well reflected in the low degradation rates under OECD test conditions, e.g. OECD 302B [24] (Zahn-Wellens Test) on inherent biodegradability. ECHA's REACH database reports 0% degradation in this test format for commercial products,[209] but values between 0 and 50% were also reported [18] (values <70% indicate a lack of biodegradability). However, the higher values in these tests were ascribed to sorption and not biodegradation.[18] In this study, we chose the OECD 302B test for inherent biodegradability based on activated sludge as it provides ideal conditions for biodegradation with a high density of the inoculum and a rather low concentration of the analyte. For comparison with data in the REACH database, we chose a commercial product of DTPMP containing minor-substituted by-products with only 1-4 phosphonate groups.[33] In contrast to the guidelines, we did not follow CO_2 or DOC evolution but determined glyphosate and AMPA concentrations over time (see Section 4.3.3.2). At the facilities of CHT Germany GmbH, Tübingen, Germany, washed activated sludge from the large WWTP Tübingen was sampled and spiked (Tü-OECD) with a commercial sample of DTPMP. Glyphosate and AMPA were not detected in the blank samples over time, but well above the LOQ in samples spiked with DTPMP (Fig. 17G). After 96 hours, the glyphosate concentrations were 1.3 and 1.7 mg/kg in the duplicates. AMPA reached concentrations of 32 and 43 mg/kg in freeze-dried samples. Glyphosate concentrations almost linearly increased further to 3.9 and 3.2 mg/kg after 408 h, whereas AMPA concentrations decreased to 21-23 mg/kg after 408 h.

All incubation experiments indicate that glyphosate is a transformation product of DTPMP in activated sludge, albeit with low yields in the batch experiments, see Section 4.4.4. Furthermore, we observed a clear dependence of the glyphosate concentration on the amount of DTPMP (see Fig. 17F). APPs are generally classified to be poorly biodegradable,[18] especially when other P sources are available,[210, 211] as generally is the case in sewage sludge. In principle, for glyphosate formation from DTPMP, an adaptation of microbial communities with enhanced microbial transformation seems possible after decades of increasing phosphonate use. Such an adaptation was reported for the artificial sweetener acesulfame,[212, 213] but also for ATMP in seawater at the discharge point of concentrates from seawater desalination plants, where phosphonates are used as antiscalants and anti-biofouling agents.[30, 214] Similarly, longer lag phases were observed for phosphonate transformation with an adaptation of the cells to these compounds, possibly due to the readying of uptake systems.[215] However, the very low transformation rates observed in OECD 302B tests on inherent biodegradability (see above) question a pronounced biotransformation of DTPMP. To investigate the transformation process in more detail, we considered two scenarios: either, glyphosate does not form but it is present in the fresh sewage sludge from herbicide applications and becomes released upon addition of DTPMP due to competitive sorption; or, abiotic or combined abiotic-biotic transformations are present as described frequently and already around 1990.[203, 216, 217]

4.4.3.2 Biotic vs. abiotic transformation

To clarify the role of abiotic reactions, we added DTPMP to sludge samples that were treated with sodium azide (PW1-DTPMP-0.75-AC). Fig. 17C and D (data in Table 9) reveal, that glyphosate and AMPA formed under these largely abiotic conditions after 72 h with concentrations reaching 50 mg/kg glyphosate and 234 mg/kg AMPA after 72 h. These concentrations were significantly higher than those in the biotic samples by factors of 10 (glyphosate) or 3 (AMPA) (PW1-DTPMP-0.75-AC vs. -BC). Some residual microbial activity is surely still present after azide treatment, but our results provide evidence that abiotic transformation is relevant for the formation of glyphosate from DTPMP. We assume that the main reason for the increase in glyphosate concentrations in abiotic samples is not an increase in its formation rate, but rather the lack of the further biodegradation of glyphosate by an active microbial community. This hypothesis is supported by incubation experiments with $^{13}C_3$-^{15}N-labeled glyphosate (G172) in activated sludge: Fig. 17E (sample PS2-G172-BC) shows that after azide treatment, only low concentrations of ^{13}C-^{15}N-labeled AMPA112 were detected. In contrast, AMPA112 concentrations were higher by a factor of about 4 under biotic conditions. The high concentrations of glyphosate in the sample impaired its quantification due to reaching the upper limit of the linear range, so Fig. 17E does not show glyphosate data. Thus, under abiotic conditions, it is possible that glyphosate accumulated. It is also possible that abiotic processes are faster than biotic transformations and thus dominate.

In contrast to the PW1 samples, similar concentrations of glyphosate and AMPA were obtained in the DTPMP concentration series (samples PS3) with 50-500 mg/kg DTPMP spiked under biotic vs. abiotic conditions, see Fig. 17F. Similar ratios AC/BC of 0.8-1.2 for a starting concentration of DTPMP ≥0.1 mg/kg are visible in Table 9. Similar ratios were also observed in experiments with $^{13}C_5$-DTPMP (PW2 samples), see Table 9, and discussion below. We presume that these differences reflect seasonal differences in microbial communities [207, 208] and climate conditions. However, there are only very few studies on glyphosate transformation in activated sludge for comparison. Poiger et al.[13] showed that over 4.5 h, glyphosate and AMPA concentrations hardly changed in an inoculum of fresh sewage sludge. Elimination rates below 10% were reported by Feng et al.[21] and the authors assumed that glyphosate inhibited microbial growth. In contrast, in acclimated sludge, a significant percentage of glyphosate was eliminated in the same study (40% in 10 h) pointing to microbial transformation.[21] However, with DTPMP likely present in the sewage sludge samples of these studies, further work is required to understand glyphosate transformation.

4.4.3.3 Transformation in activated sludge upon addition of MnO_2 and deferrization sludge

Interestingly, DTPMP transformation under abiotic conditions after azide treatment seems to proceed similar or even better compared to biotic conditions, as discussed in Section 4.4.3.2. ATMP was already shown to be transformed in the presence of MnO_x [121, 198] and glyphosate formation in aqueous solution from DTPMP was demonstrated.[17] Given the rather high concentrations of Mn present in sewage systems and WWTP (see introduction, Section 4.2) and the strong sorption of phosphonates to mineral oxides, its influence on the transformation of DTPMP seems possible. Given the rather high concentrations of Mn present in sewage systems and WWTP (see introduction, Section 4.2) and the strong sorption of phosphonates to mineral oxides, its influence on the transformation of DTPMP seems possible. To investigate this in activated sludge, we added a small amount of MnO_2 at a final concentration of 5.7 mg/kg to 35 g of fresh sewage sludge (PW1-DTPMP-0.75-MnO_2-BC) and to azide-treated activated sludge (PW1-DTPMP-MnO_2-AC) prior to DTPMP incubation (experimental details in Section 4.3.3.1 and Table 7). This results in a spiking level of 758 mg/kg MnO_2 in dry activated sludge, being above the reported concentrations of WWTP Tübingen, but below the reported concentrations of Gianico et al.[201] The molar ratio of Mn:DTPMP was ca. 0.05:1, the dry weight of activated sludge was 0.75%. Furthermore, DS from drinking water production contains high amounts of both iron and Mn and is often disposed via WWTPs (for details, see Section 4.3.2), contributing to a high Mn load in the WWTP. Presumably, different oxide forms, further denominated MnO_x. For this reason, 1 mL of DS from drinking water production was added to 35 g of activated sludge in combination without/ with azide (PW1-DTPMP-0.75-DS-BC and PW1-DTPMP-0.75-DS-AC, details in Section 4.3.3.1 and Table 7). Concentrations of incubation experiments PW1-DTPMP-0.75-BC/-AC, PW1-DTPMP-0.75-MnO_2-BC/-AC and PW1-DTPMP-0.75-DS-BC/-AC after 72 h incubation and ratios comparing AC/BC and conditions with/without MnO_x are stated in Table 10. Samples containing DTPMP and MnO_2or DS (see Fig. 18) showed a similar course of the concentration for glyphosate and AMPA compared to PW1-DTPMP-0.75-BC and -AC.

Interestingly, the ratios AC/BC for glyphosate and AMPA were clearly lower for samples PW1-0.75-MnO_2 (2.8 for glyphosate, 1.2 for AMPA) and PW1-0.75-DS (1.8 for glyphosate, 1.7 for AMPA) compared to the sample PW1-DTPMP-0.75 (9.5 for glyphosate, 1.7 for AMPA). Glyphosate concentrations under biotic conditions after 72 h were 12 g/kg with MnO_2 addition, 17 mg/kg with DS addition vs. 5 mg/kg in the samples with only DTPMP added (see Table 10). A similar trend was observed for DS samples, but glyphosate concentrations exceed concentrations in presence of MnO_2 (see Table 10). An increase of AMPA concentrations in MnO_x samples was also observed (143 mg/kg in PW1-DTPMP-0.75-MnO_2-BC, 205 mg/kg in PW1-DTPMP-0.75-DS-BC) compared to PW1-DTPMP-0.75-BC (79.5 g/kg). This demonstrated that for BC samples glyphosate and AMPA concentrations were increased by factors between 1.8-3.6 upon incubation with MnO_x (see Table 10). In contrast, factors for AC sampels were lower than 1 (except for PW1-DTPMP-0.75-DS-AC with 1.5), so a decrease in concentration compared to the PW1-DTPMP-0.75-AC samples was observed.

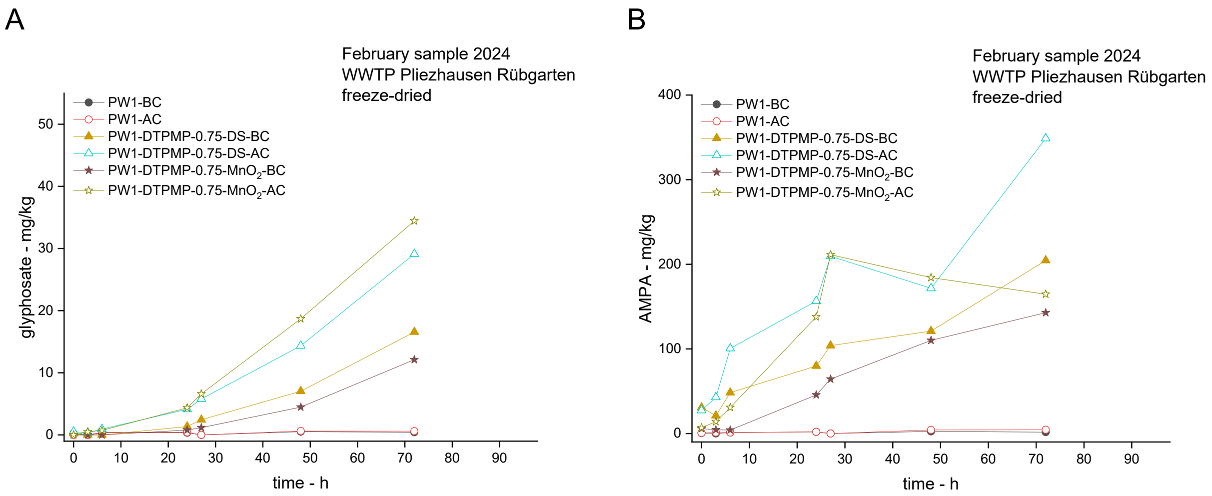


Fig. 18: Glyphosate (A) and AMPA (B) concentrations determined by CE-MS in freeze-dried samples during incubation experiments over 72 h using sewage sludge from the WWTP Pliezhausen. Experimental details in Section 4.3.3 and Table 7. Addition of DTPMP at a nominal concentration of 750 mg/kg wet sludge. We added MnO_2 (5.71 mg/kg) or 1 mL of a sludge from the deferrization of drinking water (DS) to 35 g fresh sewage sludge prior to the addition of DTPMP. Abiotic (AC) and biotic (BC) controls. All concentrations are given in mg/kg freeze-dried sludge from pooled triplicates. Glyphosate and AMPA concentrations after 72 h of incubation are summarized in Table 10.

Table 10: Concentrations and concentration ratios for glyphosate (GLP) and AMPA in freeze-dried sewage sludge after 72 h of incubation determined by CE-MS. Concentration ratios were determined for treatments without/with azide and for treatments with/without MnO_x. AC: abiotic conditions; BC: biotic conditions; MnO_x denotes samples with addition of either MnO_2 or DS= deferrization sludge from the deferrization of drinking water. Sample names as defined in Table 7. Concentration data are from pooled freeze-dried sludge from triplicates incubations. Plots for the concentration changes over time are presented in Fig. 18.

Sample name PW1-	GLP (mg/kg)	AMPA (mg/kg)	Ratio AC/BC GLP	Ratio AC/BC AMPA	Ratio with/without MnO_x GLP	Ratio with/without MnO_x AMPA
BC	0.4	1.6	1.4	2.8		
AC	0.6	4.4				
DTPMP-BC	5.3	79.5	9.5	2.9		
DTPMP-AC	49.7	233.5				
DTPMP-MnO_2-BC	12.1	142.8	2.8	1.2	2.3	1.8
DTPMP-MnO_2-AC	34.5	164.6			0.7	0.7
DTPMP-DS-BC	16.6	204.5	1.8	1.7	3.1	2.6
DTPMP-DS-AC	29.1	348.7			0.6	1.5

Nevertheless, higher glyphosate concentrations were observed under biotic conditions (factors of 1.4-9.5, Table 10). In abiotic condition experiments, glyphosate concentrations after 72 h followed the order: no MnO_x (50 mg/kg, PW1-DTPMP-0.75-AC) > MnO_2 (34 mg/kg, PW1-DTPMP-0.75-MnO_2-AC) > deferrization sludge (29 mg/kg, PW1-DTPMP-0.75-DS-AC). Order for AMPA concentrations in azide-treated samples after 72 h were as follows: deferrization sludge > no MnO_x > MnO_2. Interestingly, the differences in concentrations between biotic and abiotic samples were smaller when MnO_x was present, see Table 10. At the moment, the reasons for these observations cannot be completely explained. Additions of MnO_x may change the preferred formation pathways for both glyphosate as well as for AMPA, also indicated by the less steady AMPA formation shown in Fig. 18B compared to Fig. 17D. Furthermore, consecutive transformation pathways for glyphosate may also been changed due to the presence of higher MnO_x concentrations, as its transformation on birnessite has already been described.[218, 219] Activated sludge is a very complex system not only regarding its chemical composition but also regarding the microbial communities present as a change in MnO_x concentration can lead to changes in their activity and present communities. Finally, addition of MnO_2 or DS could also influence the analyte recoveries due to sorption especially with regard to the large amount of iron oxides present in deferrization sludge.[220] Further investigation regarding the influence of MnO_x presence on the transformation pathway of DTPMP is clearly necessary to better understand the behavior we observed in activated sludge.

4.4.3.4 In-situ formation vs. possible desorption

Negative elimination rates in WWTPs for glyphosate and AMPA were reported, comparing influent and effluent sample concentrations,[85] or considering the whole treatment process.[14] Desorption of glyphosate from particulate matter during the treatment process was discussed as a potential reason.[13, 14] Indeed, glyphosate was shown (i) to strongly sorb to sewage sludge at pH <8 (AMPA pH <7) but to fully desorb at pH >11 (AMPA pH > ca. 10), and (ii) to be present in sludge with concentrations exceeding influent concentrations by a factor of >30. [13] Throughout our batch incubation experiment, we observed a stable pH of 7 and there were no changes in glyphosate concentrations in the controls, so desorption may only have been induced competitively by DTPMP as described for the competitive sorption between phosphate and DTPMP.[221] However, with the high extraction efficiencies determined for glyphosate and AMPA (>100%, see Section 4.3.4.1) this effect is unlikely to be relevant given the strong changes in the concentration of these analytes during the incubation of DTPMP.

In order to unambiguously prove the in-situ formation of glyphosate during incubation experiments with DTPMP, we synthesized isotope-labeled $^{13}C_5$-DTPMP using ^{13}C-labeled formaldehyde (see Section S1), resulting in ^{13}C labeling of all phosphonomethyl-moieties, which are also present in the transformation products glyphosate (G169) and AMPA (AMPA111). In our synthesis, the concentration of ^{13}C-DTPMP and related by-products (with one to five phosphonate moieties) was 71.3 wt% in total with 40.5 wt% accounting for DTPMP and 30.8 wt% for DTPMP-related amino(poly)phosphonates with less than five phosphonate groups (details in Table S2-1). This mixture well reflects industrial products: for phosphonate formulations used as antiscalants in reverse osmosis applications, mixtures of DTPMP (38-65% of the total phosphorus content) and DTPMP-related APPs with a lower number of phosphonate groups were reported. The contribution of DTPMP to the sum of DTPMP and related APPs was shown to be 40-70%.[33]

With the direct CE-MS method without derivatization, we can discriminate different numbers of isotope labels present and thus differentiate between native glyphosate G168 possibly present in the raw activated sludge, singly labeled glyphosate G169 formed from ^{13}C-labeled DTPMP and G170 chosen as internal standard. Fig. 19A and B reveal that, during incubations of ^{13}C-DTPMP in fresh sludge sampled in March 2024 (PW2-13C-DTPMP-0.6-BC, PW2-13C-DTPMP-0.6-AC), labeled transformation products evolved, which unambiguously demonstrates the in-situ formation of glyphosate G169 and AMPA111 in activated sludge. The concentrations after 72 h were 5 mg/kg ^{13}C-glyphosate and 173 mg/kg ^{13}C-AMPA. Similar results were obtained under abiotic conditions (Fig. 19A and B). The AMPA:glyphosate ratio was higher in this experiment compared to PS1 and PW1 samples, which may be due to the mixture of compounds differing in the number of phosphonate groups and possibly different transformation pathways and rates. In the experiment as well as the controls, we saw a slight increase in non-labeled AMPA110, presumably from DTPMP present in the fresh activated sludge. No non-labeled glyphosate GLP168 was detected.

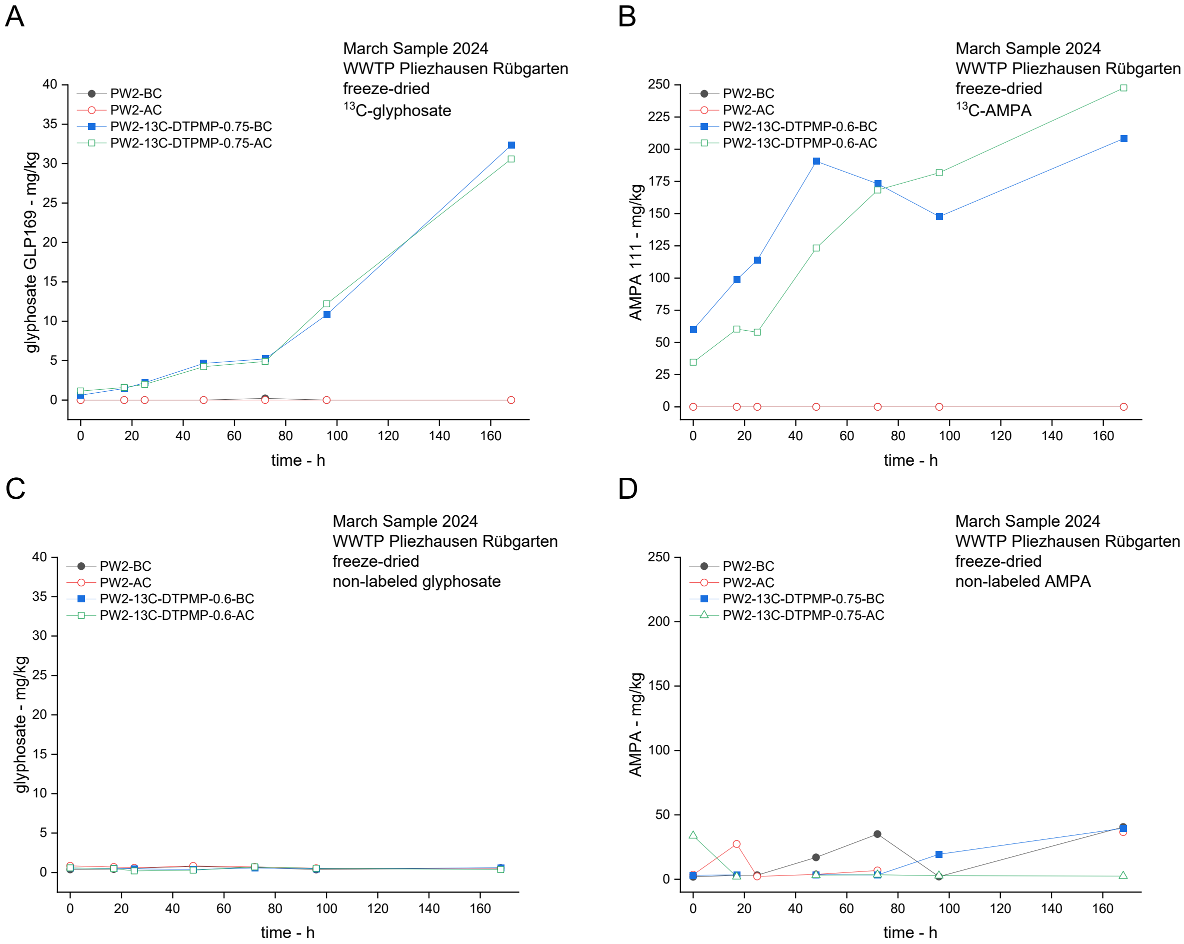


Fig. 19: Concentrations of (A) isotope-labeled glyphosate G169 and (B) AMPA111 formed from 13C-labeled DTPMP as well as (C) unlabeled glyphosate GLP168 and (D) AMPA110 in freeze-dried samples analyzed by CE-MS after incubation experiments with the synthesis mixture of $^{13}C_5$-DTPMP over 168 h using fresh activated sludge from the winter sampling (PW2 see Table 7, experimental details in Section 4.3.3). BC = biotic conditions; AC = abiotic conditions. The final concentration of $^{13}C_5$-DTPMP in sludge was 600 mg/kg; the concentration of total amino(poly)phosphonates related to DTPMP (containing the ethylene diamine moiety) was 1000 mg/kg in sludge (for synthesis and purity, see Section S2-1 and Table S2-1). All concentrations are given in mg/kg freeze-dried sludge.

4.4.4 Assessment of glyphosate concentrations in wastewater from in-situ formation from DTPMP

We used the incubation experiments with $^{13}C_5$-labeled DTPMP for a rough estimate of possible WWTP effluent concentrations of glyphosate using the weight% and mol% of labeled glyphosate formed. The synthetic mixture was not pure DTPMP, but had a similar amino(poly)phosphonate composition as reported for commercial DTPMP samples used in reverse osmosis applications (see discussion in Section 4.4.3.4).[33] We used a nominal starting concentration of about 596 mg/kg $^{13}C_5$-labeled DTPMP or 965 mg/kg total DTPMP and related amino(poly)phosphonates (details in Section S2-1). We estimated that G169 formed after 72 h of $^{13}C_5$-DTPMP incubation in a range of 0.01 to 0.02 mol% under both biotic and abiotic conditions, see Table 11. AMPA111 formed in the range of 0.6 to 1.1 mol%. Residual DTPMP present in the sludge samples does not seem to have been relevant for the formation of glyphosate as no unlabeled glyphosate was detected in the experiments with $^{13}C_5$-labeled DTPMP (see Fig. 19C). Concentrations of unlabeled AMPA, possibly formed from residual DTPMP, remained low over the course of the experiment and fluctuated between <LOD and <50 mg/kg (see Fig. 19D). Similarly, no glyphosate and AMPA were detected in BC and AC controls of the other experiments (see Fig. 17).

Table 11: Labeled glyphosate and AMPA concentrations and yields for the formation in activated sludge from the incubation of a mixture of amino(poly)phosphonates formed during synthesis after 72 h (PW2-13C-DTPMP-06-BC and AC, see Table 7). Concentrations determined by CE-MS. No unlabeled glyphosate and only very small amounts of unlabeled AMPA from possible residual DTPMP were observed in the corresponding controls (see Fig. 19C-D). Using the purities stated in Table S2-1, the nominal concentration of $^{13}C_5$-DTPMP spiked to activated sludge was 0.6 g/kg, the total concentration of all $^{13}C_x$-labeled amino(poly)phosphonates in the product mixture from synthesis with an ethylenediamine moiety was 1.0 g/kg. We determined 0.75% of the weight of activated sludge to account for the dry weight. This value was used to calculate the final spiking concentrations for the model calculations.

Experiment	**Spiked DTPMP**	**Concentration in freeze-dried samples after 72 h**		**Yields of glyphosate and AMPA formation (mol%)**		**Yields of glyphosate and AMPA formation (weight%)**	
	$^{13}C_5$-DTPMP g/kg	G169 mg/kg	AMPA111 mg/kg	G169 mol%	AMPA111 mol%	G169 weight%	AMPA111 weight%
BC	80.0	5.22	173.19	0.022	1.12	0.007	0.217
AC	80.0	4.90	168.37	0.021	1.09	0.006	0.211
	$^{13}C_x$-amino-(poly)phos-phonates g/kg	G169 mg/kg	AMPA111 mg/kg	G169 mol%	AMPA111 mol%	G169 weight%	AMPA111 weight%
BC	133.3	5.22	173.19	0.011	0.56	0.004	0.130
AC	133.3	4.90	168.37	0.011	0.55	0.004	0.126

The same calculations were made for the activated sludge incubation experiments PW1 and PS3 using pure DTPMP, the results are summarized in Table S2-2. The formation of glyphosate in DTPMP incubations ranged from 0.017 to 0.040 mol% in biotic samples and 0.023 to 0.167 mol% in abiotic samples. AMPA formed in the range of 0.402 to 1.72 mol% in biotic samples and in the range of 1.18 to 1.46 mol% in abiotic samples. These numbers are slightly higher than those observed for $^{13}C_5$-labeled DTPMP, which reflects different reaction rates in sludge samples from different sampling campaigns as well as differences in the spiked DTPMP (pure vs. synthetic mixture).

The formation of glyphosate and AMPA in activated sludge is lower than in aqueous model systems with manganese dioxide as a reaction partner.[17] Maximum production amounts of 0.16 mol% glyphosate and 10.1 mol% AMPA were observed under oxic conditions. Differences can be expected from matrix effects in activated sludge and additional biotic processes, which are relevant for the consecutive glyphosate transformation, see Fig. 17E.

We used these semi-quantitative data on glyphosate formation from DTPMP for a rough estimate whether this transformation process can aid in explaining the WWTP effluent concentrations observed in Europe. Our estimate was based on 1,500-2,500 tons of DTPMP used in Germany as discussed in the introduction. We assumed 100% to be relevant for wastewater as discussed by Groß et al.[193] Using the weight% in Table 11 from the experiment with $^{13}C_5$-DTPMP, 60-160 kg glyphosate and 1950-5410 kg AMPA could form per year in activated sludge. 10 billion m³ of wastewater are treated annually in Germany.[195] With an elimination rate of 80% for glyphosate,[13] concentrations of 0.001-0.003 µg/L glyphosate and 0.03-0.1 µg/L AMPA are calculated for WWTP effluents. For comparison, in our previous publication,[5] we estimated the concentration of glyphosate expected to be present in WWTP effluents, from its sales numbers for non-occupational use (10-100 tons), loss rates of 1% from residential areas, 10 billion m³ wastewater in Germany and glyphosate elimination rates of 80%, to be 0.002-0.02 µg/L.

Both estimates are below the range of concentration data published in literature (country, year, range, number of WWTPs sampled): median 0.34 µg/L (CH, 2016, 0.06-3.8 µg/L; 42),[88] median 0.16 µg/L (CH 0.047-0.58 µg/L in 2009 sampled over one year, 1),[13] mean 1.6 µg/L (NL, range not given, in 2010, 25),[94] mean of 0.55 µg/L (DE, 0.076-5.4 µg/L, sampled over one year in 2018 in the WWTP Adelzhausen, data kindly provided by the Bayerisches Landesamt für Umwelt, Germany). However, for the relevance of DTPMP for glyphosate formation, several potential factors have to be considered in addition to the data used in our estimate here: (i) Our incubation experiments were batch experiments compared to a WWTP which can be regarded a flow-through reactor, so differences in reactions rates are possible. (ii) Sludge retention times can be larger than the time span investigated in the incubation experiments. (iii) Additional, local sources for DTPMP other than household products may be present such as industry for food production, textile industry or reverse osmosis treatment for drinking water production, etc. (iv) Glyphosate formation is possible already in the sewer system as indicated by elevated influent concentrations all over the year.[13] (v) Finally, transformation and elimination rates can be expected to largely differ between WWTPs but also between seasons and may occur at different points in the sewer system or WWTP.

4.5 Conclusion

In this study, we proved that both glyphosate and AMPA are transformation products of DTPMP when spiked into activated sludge. Their in-situ formation was demonstrated using ^{13}C-labeled DTPMP. The formation of glyphosate and AMPA was observed both in fresh activated sludge and in incubations following the OECD 302B guideline on inherent biodegradability. As the formation of glyphosate and AMPA was higher or similar in controls spiking DTPMP to activated sludge after azide treatment or the addition of MnO_x, abiotic transformation processes might dominate in activated sludge, possibly involving MnO_2 as indicated by Röhnelt et al.[17]

The results presented here can aid in explaining many observations for glyphosate and AMPA in wastewater as the use of DTPMP in household applications is rather constant over the year explaining the detection of the compounds in the WWTP all over the year,[13] also when rain-driven input is negligible [119] and also for WWTPs connected to a separate sewer system.[87] Similarly, glyphosate and AMPA concentration patterns in rivers with rather constant mass fluxes, and their relation to pharmaceuticals but not to other herbicides may be better understood as well as the contrasting findings in Europe and USA, where phosphonates are hardly used in laundry products.[5] Negative elimination rates [14] may be understood from the in-situ formation in sludge. However, as shown with our estimate on wastewater concentrations, the low in-situ yields of formation observed in the batch laboratory experiments would not yet be sufficient to explain the high concentrations observed in wastewater treatment and rivers.

To fully understand the impact of APPs on surface water contamination, further research should focus on the understanding of the abiotic reactions, on reactions in flow-through reactors to better simulate conditions in the WWTP, formation rates in sludge from different WWTPs and in the sewer networks. Furthermore, investigation of the correlation between influent and effluent concentrations of DTPMP, glyphosate and AMPA and possibly further transformation products and concentrations in receiving rivers.

5 Relevance of a wastewater treatment plant on the spatial variability of glyphosate, AMPA and DTPMP in the receiving river before and after its closure

5.1 Abstract

Glyphosate and aminomethylphosphonic acid (AMPA) are detected in surface waters worldwide, but their analysis mostly focused on the dissolved concentrations despite their strong tendency for sorption to minerals. Wastewater treatment plants (WWTPs) have been identified as a major source of glyphosate and AMPA pollution to their receiving rivers, mostly with the assumption that glyphosate enters WWTPs via the sewer system after application in urban areas. Recent studies stress the relevance of diethylenetriamine penta(methylene)phosphonic acid (DTPMP) as their precursor leading to an in-situ formation e.g. in activated sludge. In this study we identified the effluent of the WWTP Heroldsberg as the dominant source of glyphosate, AMPA, and DTPMP in the receiving stream, the Gründlach. High glyphosate and AMPA concentrations were found in the supernatant of the activated sludge from the aeration tank (9.6 µg/L glyphosate, 31.2 µg/L AMPA). Glyphosate and AMPA concentrations were very high and increased upstream vs. downstream of the WWTP in the Gründlach both for dissolvend concentrations (glyphosate <LOD to 4.5 µg/L, AMPA by a factor of 30) and in sediment (glyphosate <LOD to 0.8 mg/kg, AMPA 0.5 to 11.9 mg/kg by a factor of 28). Similarly, DTPMP concentrations in sediment were higher than reported elsewhere with 89 mg/kg at the discharge point. Decreases in these concentrations along the river course indicated attenuation processes, including transformation, dilution, sorption and, for glyphosate a possible formation from DTPMP and for AMPA, possibly also a formation from glyphosate and/or DTPMP. After the WWTP shutdown, glyphosate concentrations in water and sediment rapidly decreased to values near the LOQ, while AMPA concentrations peaked during the shutdown phase but then decreased to 0.28-2.3 µg/L and 0.7-3.8 mg/kg, respectively, after seven months over the entire river stretch monitored. Similar observations were made for sediments, demonstrating that the river quickly recovers despite the high contamination during operation.

5.2 Introduction

Wastewater treatment plants (WWTPs) were frequently identified as a source of glyphosate and aminomethylphosphonic acid (AMPA) in surface waters.[5, 6, 11, 93] Using long time series of glyphosate and AMPA concentrations, Schwientek et al. argued that herbicide application cannot explain the rather constant mass fluxes in rivers impacted by WWTP effluent and the lack of correlation to the patterns of other herbicides.[5] Based on an intense evaluation of data from surface water monitoring, the authors defined criteria for an overlooked source of glyphosate and AMPA and identified aminopolyphosphonates (APPs) commonly used e.g. in laundry detergents as a possible precursor.[5] In Section 4 we demonstrated that the transformation of the APP diethylenetriamine penta(methylene phosphonic acid) (DTPMP) in activated sludge indeed yields glyphosate and AMPA. This transformation may better explain the rather constant input, high urban loads,[5] the almost ubiquitous presence of glyphosate in WWTP influent, sewage sludge and WWTP effluent [6, 13, 87] as well as negative elimination rates.[6, 14, 85]

The impact of WWTPs on glyphosate and AMPA concentrations in receiving rivers was only studied looking at water concentrations. However, given the tendency of aminophosphonates to adsorb to sediment, activated sludge and soil minerals,[19, 35, 52, 222] the analysis of sediment samples can provide more comprehensive insights into the origin and occurrence of these compounds in surface water. The need to include sediment analysis in studies of glyphosate's and AMPA's fate in aquatic ecosystems is further stressed by sediment cores forming archives of these substances. For instance, as shown by the analyses elevated concentrations down to 20 cm of glyphosate and AMPA [73] were found in sediment cores of the Anlagensee (Tübingen, Germany) ranging from 0.05-0.08 mg/kg for glyphosate and 1.21-2.10 mg/kg for AMPA. Similarly, DTPMP was detected in a sediment core of the Rhine down to 66 cm depth with concentrations ≤1 mg/kg.[22] Currently, there is no knowledge on how the strong sorption of the aminophosphonates may influence their attenuation and transport. Kolpin et al. summarized data for AMPA and glyphosate concentrations for eleven WWTPs in the USA with one sample upstream and two downstream samples at various distances to the WWTP. AMPA was frequently detected in WWTP effluent and showed elevated concentrations downstream to the WWTP with a high detection frequency of >78%, clearly controlled by the concentrations in the effluent. In contrast, glyphosate was detected in less than 1/3 of all WWTP effluents but concentrations and detection frequencies were similar up- and downstream to the WWTP,[12] likely impacted by an intense agricultural use.

Very few publications are available on DTPMP concentrations in surface waters influenced by WWTP effluent.[223] A recent study by Rott et al. demonstrated that DTPMP does indeed reach the receiving waters and can be detected in the sediment of these rivers.[22] Furthermore, they reported a significant effect on the influence of WWTP effluent on phosphonate concentration in the receiving river previously not impacted by WWTP effluent, with an increase of phosphonate concentration by a factor 8 comparing upstream vs. downstream concentrations. In contrast, concentrations did not increase significantly (factor of 1.9) if the receiving river was already influenced by WWTP effluent.[22]

The in-situ formation of glyphosate and AMPA from DTPMP in activated sludge (see Section 4) necessitates further research on the correlation of these compounds in receiving rivers as reported elimination rates of DTPMP and glyphosate are similar with 80-95% via adsorption to sludge in WWTPs with phosphate removal.[18, 21, 75] This suggests that DTPMP is not only retained in the WWTP via adsorption to sludge but becomes (partially) transformed with a concomitant release of transformation products. In sediments, DTPMP and its transformation products may become enriched due to sorption and the long half-lives, e.g. of 74 days reported in sediment for glyphosate and 395 days in water for DTPMP.[23] Given the chemical relationships between the compounds DTPMP and glyphosate and AMPA, this study focuses on their input from a WWTP and the changes in their concentrations in the river surface water and sediment phase. It is hypothesized that the WWTP effluent constitutes the main source of DTPMP, AMPA and glyphosate in the Gründlach. Moreover, it is assumed that due to sorption, concentrations of compounds will remain elevated in surface water for a certain period of time after the WWTP shutdown. The attenuation of these compounds is studied using a longitudinal profile of concentrations along a 7.5 km river reach. In addition, the impact of shutting down this WWTP on surface water and sediment concentrations of DTPP, AMPA and glyphosate is investigated to understand how quickly the system can recover following a step-function-like cessation of compound inputs.

5.3 Materials and Methods

5.3.1 Chemicals

All chemicals were used without further purification.

Ultra-pure water (≥18 MΩcm^{-1}) was obtained using an ELGA-Veolia PURELAB Classic system (Celle, Germany) or a Milli-Q EQ 7000 pure water generator (MerckMillipore, Darmstadt, Germany) and used throughout the study.

Phosphonates: DTPMP standard was purchased from Zschimmer & Schwarz Chemie (Lahnstein, Germany) as free acid powder under the name “Cublen D 900 GR” with an active acid content of >90% (CAS: 15827-60-8). The purity was verified by ^{1}H and ^{31}P-NMR.

Chemicals for extraction: From Sigma Aldrich (Steinheim, Germany) we bought sodium hydroxide (≥98%, pellets), sodium phosphate heptahydrate dibasic ($Na_2HPO_4 \cdot 7\ H_2O$) (≥99%) and nitrilotriacetic acid (≥99). Hydrochloric acid (37%, technical grade) for acidification of sample extracts was purchased from Brenntag (Reading, PA, USA).

Chemicals for analysis: Glyphosate and AMPA standards for the different analytical methods were purchased from the following companies: **CE-MS and LC-MS-1:** AMPA-^{13}C-^{15}N (AMPA112) was from Toronto Research Chemicals (Toronto, Canada) and glyphosate-2-^{13}C-^{15}N (G170) (≥99%) from Sigma Aldrich (Steinheim, Germany). **LC-MS-2:** Glyphosate-^{13}C-^{15}N (G170) and AMPA-^{13}C-^{15}N-methylene-D_2 (AMPA114) were bought from Cambridge Isotope Laboratories (Andover, USA) and used as internal standards.

Chemicals for mass spectrometry were purchased from the following companies with LC-MS grade purity: **CE-MS and LC-MS-1:** Ammonium hydroxide solution (ca. 25%) was purchased from Sigma Aldrich (Steinheim, Germany), acetonitrile from Honeywell (Seelze, Germany) and VWR Chemicals (Darmstadt, Germany), methanol from Th. Geyer (Renningen, Germany), formic acid, isopropanol and water from Merck (Darmstadt, Germany). Caffeine (anhydrous, ≥98.5%) from Carl Roth (Karlsruhe, Germany) was used as an internal standard to control the stability of the ionization process. **LC-MS-2:** Methanol (LC-MS grade) and acetonitrile (LC-MS grade) were from Carl Roth (Karlsruhe, Germany), ammonium carbonate from Sigma Aldrich (Steinheim, Germany). Aqueous sodium hydroxide (0.1 M) solution was obtained from Applichem Panreac ITW Companies (Darmstadt, Germany).

Chemicals used for the derivatization of DTPMP via methylation for **LC-MS-1** were bought from the following companies: trimethylsilyldiazomethane (1.8-2.4 M in hexane) from Thermo Fisher Scientific (Schwerte, Germany), and aqueous sodium hydroxide (1 M) solution from Agilent Technologies (Santa Clara, Ca, USA). For the derivatization of glyphosate and AMPA analyzed by **LC-MS-2**, fluorenylmethyloxycarbonyl chloride (FMOC-Cl) was purchased from Sigma Aldrich (Steinheim, Germany) and sodium tetraborate and ethylenediaminetetraacetic acid from Merck (Darmstadt, Germany).

5.3.2 Samples and sampling locations

The WWTP Heroldsberg is located approx. 10 km northeast of City of Nuremberg, Germany. Sediment and aqueous samples of the receiving river, the Gründlach, were collected along an approx. 7.5 km long reach to record changes in the concentrations of the aminophosphonates along a longitudinal profile. In order to investigate the impact of the WWTP effluent on the surface water and sediment concentrations, one sampling site was located upstream (S0) and four sampling sites were located downstream (S1 to S4) of the WWTP Heroldsberg (see Fig. 20). Additionally, samples were taken from a tributary creek not impacted by WWTP effluent from this WWTP. The wastewater influx of the WWTP is 2,200 m³ during dry weather and 6,480 m³/d during wet weather conditions. It has a purification capacity of 97.2% for biodegradable carbon compounds, 94.7% for chemically oxidizable carbon compounds, 90.9% for phosphorous and 84.2% for nitrogen compounds. The Heroldsberg WWTP consists of a mechanical treatment step followed by a two-step biological treatment, preceding denitrification and chemical phosphate elimination.[224] Sampling points, dates, GPS coordinates, distance to the WWTP Heroldsberg for sediment and aqueous samples are listed in Table 12.

The activated sludge sample from the aeration tank was obtained using a plastic beaker and poured into a 1 L polypropylene bottle.

Fig. 20: Sampling sites at the Gründlach near Heroldsberg, Germany. Sampling locations are numbered as follows: S0 upstream of the WWTP Heroldsberg, S1 directly at the WWTP discharge entrance and S2-4 downstream to the WWTP (S1 = 0 km; S2 = 2.91 km, S3 = 4.79 km, S4 = 7.48 km). The tributary creek is not influenced by WWTP discharge.

The WWTP was in full operation until October 18th, 2023. That day, the wastewater flux was switched to the WWTP Nuremberg.[225] However, the tanks and basins of the WWTP Heroldsberg were rinsed several times with groundwater until approx. November 5th, 2023.

Samples were taken during full operation, during rinsing and several months after full closure as summarized in Table 12. After sampling, the specimens were stored at -20 °C until derivatization or freeze-drying for extraction. Sludge and sludge supernatant samples were centrifuged immediately after arrival at the laboratory in Tübingen. The supernatant was stored at -20 °C and transported to the Zweckverband Landeswasserversorgung Langenau for analysis.

Glyphosate and AMPA extraction from sediment: The protocols described in a previous publication were followed [44] and adapted to the sample amount chosen. Briefly, sediment samples were freeze-dried, sieved to a particle size <63 µm and extracted. The extraction medium was an aqueous solution of a mixture of 50 mM NaOH and 50 mM Na_2HPO_4 containing 400 µg/L of the added isotope-labeled internal standards AMPA112 and G170.

DTPMP extraction by competitive complexation: The extraction protocol of Armbruster et al. was followed,[33] using 150 mg (± 0.1%) of the freeze-dried sediment samples. When less sample was available, all extraction steps were downsized to maintain a constant liquid:solid ratio of 15. After the addition of 2.25 mL ultra-pure water, the samples were shaken on an overhead shaker for one hour to ensure wetting. Then, 2.25 mL of the aqueous extraction medium containing 0.5 M NaOH and 0.1 M nitrilotriacetic acid were added, the samples were sonicated for one hour at 50 °C and then centrifuged at 1,520 g for 10 min. 2 mL of the supernatant were sampled while the remaining supernatant was discarded. The extraction was repeated once. We added 500 mg cation exchange resin (AmberChrom 50Wx8 100-200 (H), Thermo Scientific, Schwerte, Germany) to each extract of 2 mL and the resulting suspensions were shaken on an overhead shaker for one hour to remove metal cations. The supernatants were then filtered using 1 mL-syringes with Chromafil Xtra RC-20/13 filters (Macherey-Nagel, Düren, Germany) with a pore size of 0.2 µm and a membrane diameter of 25 mm. Finally, the samples were neutralized to a pH of 7-9 using an aqueous 1 M NaOH solution. The phosphonates were then derivatized according to a protocol by Wang et al. [65] using trimethylsilyldiazomethane as derivatization agent (see also [44]). For quantification, standard addition was used to omit matrix effects as isotope-labeled standards for DTPMP are not commercially available. For this, 25 µL of the first and second neutralized extract were combined in an amber glass vial. A variable volume of the aqueous DTPMP stock solution (1 mg/L) was mixed with ultra-pure water to a final volume of 10 µL. These 10 µL were added to the combined sample extracts. Final concentrations of DTPMP added were 20, 40 and 80 µg/L for samples up to October 17, 2023, and for later samples 200, 600, and 800 µg/L (7.5 mg/L stock solution) to increase the slopes of the calibration functions. The amount of methanol added was reduced by 10 µL due to the additional volume from standard addition.

Table 12: Sampling points and their GPS-coordinates with their distance to the WWTP Heroldsberg along the receiving river Gründlach together with the sampling dates for sediment and aqueous samples.

sampling points	GPS coordinates (Lat., Lon.)	distance from WWTP (km)	sampling times (sediment)	sampling times (aqueous)
S0	49.522778, 11.144333	approx. 100 m upstream	27 Sep., 2023	27 Sep.,2023
			16 Oct., 2023	-
			13 May, 2024	13 May,2024
S1	49.523306, 11.142806	0	27 Sep., 2023	27 Sep.,2023
			15 Oct., 2023	-
			16 Oct., 2023	-
			04 Oct., 2023	04 Nov.,2023
			08 Nov., 2023	08 Nov.,2023
			14 Nov., 2023	14 Nov.,2023
			27 Nov., 2023	27 Nov.,2023
			07 Feb., 2024	-
			13 May, 2024	13 May,2024
S2	49.525333, 11.118389	2.91	27 Sep., 2023	27 Sep.,2023
			16 Oct., 2023	-
			04 Nov., 2023	04 Nov.,2023
			08 Nov., 2023	08 Nov.,2023
			-	14 Nov.,2023
			27 Nov., 2023	27 Nov.,2023
			07 Feb., 2024	07 Feb.,2024
			13 May, 2024	13 May,2024
S3	49.525139, 11.104683	4.79	27 Sep., 2023	27 Sep.,2023
			15 Oct., 2023	-
			16 Oct., 2023	-
			18 Oct., 2023	-
			04 Nov., 2023	04 Nov.,2023
			08 Nov., 2023	08 Nov.,2023
			-	14 Nov.,2023
			27 Nov., 2023	27 Nov.,2023
			07 Feb., 2024	07 Feb.,2024
			13 May, 2024	13 May,2024
S4	49.519472, 11.081583	7.48	27 Sep., 2023	27 Sep.,2023
			16 Oct., 2023	-
			04 Nov., 2023	04 Nov.,2023
			08 Nov., 2023	08 Nov.,2023
			-	14 Nov.,2023
			-	27 Nov.,2023
			07 Feb., 2024	-
			-	13 May,2024
Tributary creek	49.525778, 11.133056	-	16 Oct., 2023	-

5.3.3 Analytical Methods

In this study, we used capillary electrophoresis-mass spectrometry (CE-MS) to determine glyphosate and AMPA concentrations and LC-MS for DTPMP analysis after derivatization with trimethylsilyldiazomethane (LC-MS1). To quantify glyphosate and AMPA in aqueous samples, LC-MS was used after derivatization with FMOC (LC-MS2) to reach the LOQs required.

Capillary electrophoresis–mass spectrometry (CE-MS): The CE-MS method was conducted as described by Engelbart et al.[44] Bare fused silica capillaries with an inner diameter of 50 µm and a length of 75 cm from Polymicro Technologies (Phoenix, USA) were used throughout the study.

LC-MS-1 with trimethylsilyl diazomethane derivatization and **LC-MS-2 with FMOC derivatization** were performed as described in a previous publication.[44]

5.4 Results and Discussion

In order to show the relevance of a WWTP for the release of the phosphonates DTPMP and its transformation products AMPA and glyphosate, these phosphonates were analyzed in wastewater samples and in river and sediment samples upstream and downstream of the WWTP along a longitudinal profile in the receiving river. In addition, we investigated, how these concentrations changed over seven months following the closure of the WWTP.

5.4.1 Impact of the WWTP on phosphonate concentrations in the receiving river

As shown in Fig. 21A, very high concentrations of glyphosate and AMPA were detected in the supernatant of the aeration tank of the WWTP Heroldsberg reaching 9.6 µg/L for glyphosate and 31.3 µg/L for AMPA. For comparison, Venditti et al. observed significantly lower concentrations in the WWTP influent (0.10 µg/L glyphosate and 2.3 µg/L AMPA) and effluent (0.13 µg/L glyphosate and 4.1 µg/L AMPA) of a WWTP in Luxembourg.[14] These values not only indicate a negative elimination rate for this WWTP, but are also approximately ten times lower than the concentrations observed in the WWTP Heroldsberg. Poiger et al. showed concentrations of approximately 0.1-1.5 g/L for glyphosate and 0.5-2 µg/L for AMPA in the secondary effluent of a Swiss WWTP. Concentrations in the final effluent were 0.16 µg/L on average (0.047-0.58 µg/L). In the same WWTP, concentrations observed in activated sludge samples were always 1-2 orders of magnitude higher than corresponding concentrations in secondary effluent,[13] indicating an even higher glyphosate and AMPA load on activated sludge to be expected in the WWTP Heroldsberg. In a further study, Poiger et al. found average glyphosate concentrations of 2-3 µg/L in the primary effluent, which decreased to 0.05-0.58 µg/L in the final effluent.[13] At a German WWTP, monthly sampling showed a median glyphosate effluent concentration of 0.55 µg/L (range <LOD to 5.4 µg/L) and an AMPA concentration of 1.35 µg/L (range 0.05-5.0 µg/L), data kindly provided by the Bayerisches Landesamt für Umwelt, Germany. Similarly, in the Netherlands, average concentrations in WWTP effluents along the Meuse and its tributaries in 2010 were 1.6 µg/L glyphosate (up to 29.2 µg/L) and 3.5 µg/L AMPA (up to 50 µg/L).[94] In the USA, Battaglin et al. detected glyphosate in only 1 of 11 samples from WWTP effluents, with a median concentration of <0.02 µg/L (maximum 0.30 µg/L) and AMPA in 9 of 11 samples, with a median concentration of 0.45 µg/L (maximum 2.54 µg/L).[84] With the very high concentrations observed for the WWTP Heroldsberg, specific local sources for glyphosate may be assumed.

Fig. 21A also shows the concentrations of the aminophosphonates in the receiving river Gründlach. Upstream to the WWTP, glyphosate was neither detected in water (LOD = 0.01 µg/L) nor in sediment (LOD = 63 µg/kg) (see Fig. 21A and B). AMPA concentrations were 0.5 µg/L and 0.5 mg/kg. Concentrations of glyphosate strongly increased to 4.5 µg/L and 0.8 mg/kg at the discharge point of the WWTP into the water of the receiving river Gründlach. For AMPA, a similarly strong increase to 15.9 µg/L and 11.8 mg/kg was observed. This clearly demonstrates the WWTP to be the dominant source for these compounds, corroborating the findings by Poiger et al. for surface waters in Switzerland.[6] Sediment and water concentrations in the Gründlach were high when compared to other studies: Concentrations from a Swiss WWTP effluent [13] were 12 times lower than the concentrations measured directly at the discharge point of the WWTP Heroldsberg for both analytes despite dilution in the receiving river. In Argentina, 13 km downstream of a WWTP the mean sediment concentration of glyphosate was 0.6 mg/kg (up to 1.9 mg/kg), which is lower than in the Gründlach, while water concentrations were higher with a mean of 35.2 µg/L glyphosate (up to 125 µg/L) and 0.6 µg/L AMPA (up to 2.2 µg/L).[226] Another Argentinean study showed glyphosate concentrations in sediments ranging from 0.005-0.221 mg/kg glyphosate (detected in 66% of samples) and 0.005-0.235 mg/kg AMPA (detected in 89% of samples).[227] In the USA, median concentrations of 0.01 mg/kg glyphosate and 0.02 mg/kg AMPA were reported,[84] both far below the concentrations detected in the sediment of the Gründlach at the sampling positions less than 5 km from the point of discharge of WWTP Heroldsberg. Both analytes are also detected in the same range in German rivers as described for US rivers by Battaglin et al., with the concentrations ranging from 0.01-0.07 mg/kg for glyphosate and from 0.03-0.1.25 mg/kg for AMPA in upper sediment layer.[73, 84]

Reanalyzing data from Wimmer et al. [73] revealed that only rivers impacted by WWTP effluent had elevated sediment concentrations as high as 0.01-0.06 mg/kg for glyphosate and 0.05-0.53 mg/kg for AMPA. The highest concentrations were observed in the small artificial lake Anlagensee (Tübingen, Germany) fed by a river impacted by a WWTP, reaching AMPA concentrations of up to 2.1 mg/kg [73], which were still lower than those in the Gründlach near the WWTP Heroldsberg.

The impact of WWTPs on glyphosate and AMPA concentrations was reported several times.[5, 94, 228] For example, concentrations of glyphosate and AMPA in streams across Switzerland ranged from a median of 0.11 µg/L to 0.20 µg/L and were compared to concentrations in effluents from WWTPs with median values of 0.38 µg/L and 1.3 µg/L, respectively.[6] Poiger et al. discussed that for 13% of the WWTPs in the study, WWTP effluent was the predominant source of glyphosate (>90%), in 43% it was significant with 20-90% input explained but negligible for the remaining 42% of the WWTPs.[6] The authors presumed that the relevance of the WWTP as a source for glyphosate was mainly related urban herbicide applications. Similarly, the contribution of non-agricultural uses of glyphosate was discussed as a possible explanation for the ubiquitous occurrence of glyphosate in wastewater.[6] Botta et al. demonstrated the relevance of non-agricultural impact on the annual glyphosate load, with an increase from 0.5 µg/L glyphosate in an agricultural area to 1.5 µg/L glyphosate in an urban area.[9] They showed a trend of decreasing glyphosate concentrations along the longitudinal profile downstream of a WWTP. AMPA concentrations also increased between the agricultural and urban area from 0.1 µg/L to 0.5 µg/L, but further increased due to a WWTP to up to 1.44 µg/L, albeit no clear decreasing trend as for glyphosate was observed with distance to the WWTP.[9] Corroborating an urban input, Tauchnitz et al. [93] detected glyphosate in 63% of analyzed samples at an urban sampling site outside the typical agricultural application times. A study by Kolpin et al. showed a roughly two-fold increase in the frequency of glyphosate and

AMPA detection between samples up- and downstream of WWTPs, indicating an important contribution of WWTP effluent on the concentrations of both glyphosate and AMPA in receiving rivers.[12] In their study, Desmet et al. stated that WWTP effluent was the source of 29% of glyphosate and 12% AMPA in the river Meuse, using data from WWTPs draining directly into the Meuse (neglecting a possible input from WWTPs via tributaries).[40]

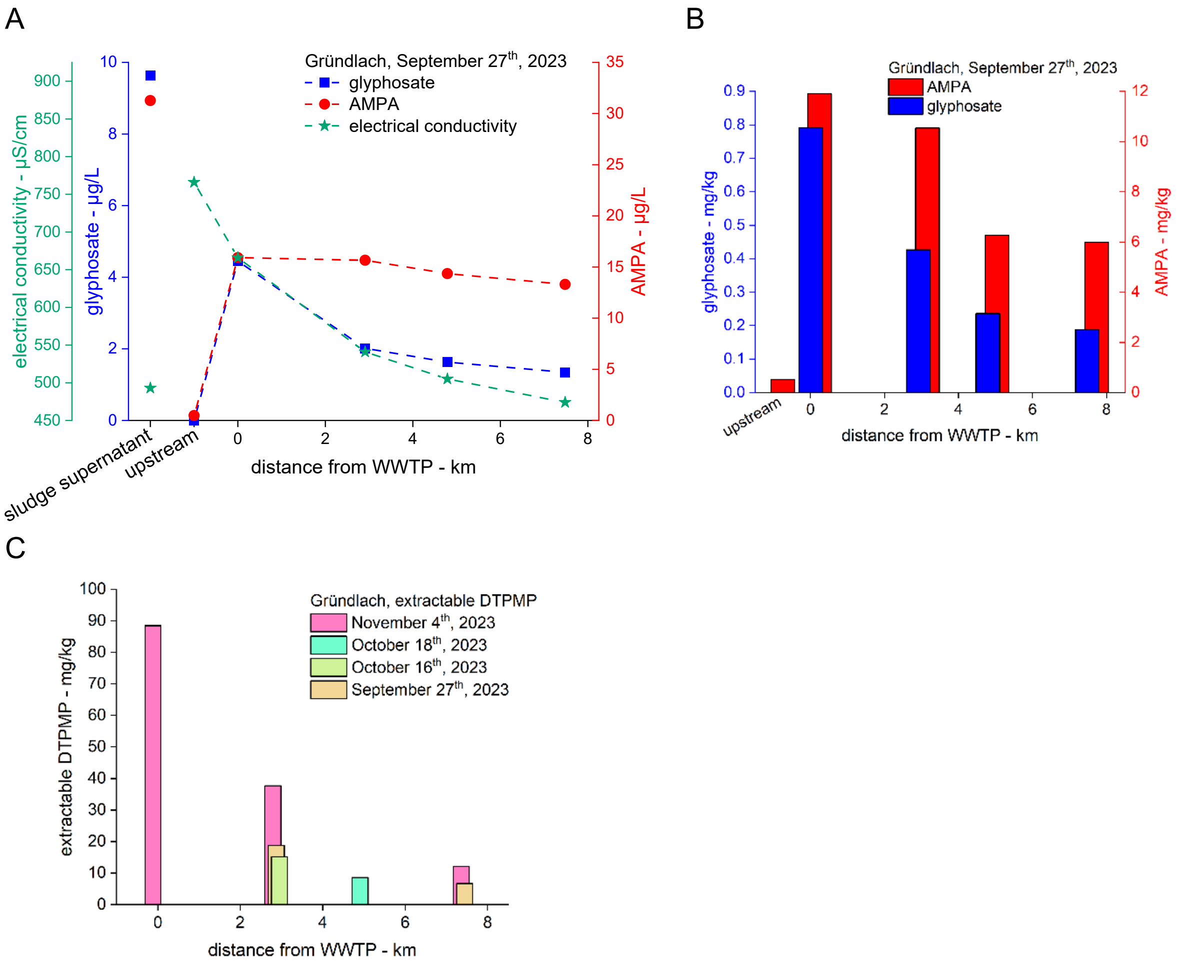


Fig. 21: Longitudinal profiles of glyphosate, AMPA and DTPMP concentrations from the receiving river Gründlach: (A) concentration in µg/L in river water and aqueous supernatant from the aeration tank. (B) glyphosate and AMPA concentrations and (C) extractable DTPMP concentration in mg/kg in the upper sediment layer (particle size ≤63 µm). Sampling date for (A) and (B): September, 27th 2023 with WWTP Heroldsberg in operation. Sampling dates for C: September, 27th, October 16th, October 18th and November, 4th 2023. Distance from WWTP Heroldsberg: 100 m upstream, directly at the discharge point into the Gründlach, 2.9 km, 4.78 km and 7.5 km downstream, see Fig. 20.

For some WWTPs and receiving rivers glyphosate concentrations were discussed to be too high to be explained by herbicide applications.[5] Instead, APPs were discussed to be possible precursors for glyphosate and AMPA. This was proven recently when incubating DTPMP in sewage sludge (see Section 4). With DTPMP used in laundry products in Europe but hardly in the USA, the large difference in WWTP effluent, river water and river sediment concentrations of glyphosate and AMPA between the continents may be explained. This study is the first one that looks at the co-occurrence of glyphosate and AMPA with their precursor DTPMP to better understand connections between their concentrations. In order to better understand the relevance of DTPMP for glyphosate and AMPA input, we also determined DTPMP concentrations in the sediment of the receiving river Gründlach (shown in Fig. 21C), which were expected to also be related to the very high concentrations of glyphosate and AMPA and thus elevated compared to other reports. DTPMP concentrations are stated as extractable DTPMP due to its strong sorption resulting in calculated extraction efficiencies below 50% (data not shown).

No DTPMP could be detected in sediment upstream to the WWTP Heroldsberg (LOD = 3.6 mg/kg). At the point of discharge of the WWTP, we detected a very high concentration of 89 mg/kg extractable DTPMP on the 4th November, 2023, while earlier sampling dates were not quantifiable at this sampling site due to too low sample amounts available. For comparison, a similar study by Rott et al. investigated phosphonate concentrations in the sediment and suspended particles of two receiving rivers, Körsch and Neckar (both in Stuttgart, Germany) with extractable DTPMP concentrations ranging from 0.36-1.9 mg/kg downstream a WWTP,[22] which is clearly lower than those observed in our study. Interestingly they demonstrated a significant effect on phosphonate concentration only in the receiving river previously not impacted by WWTP effluent (see Section 5.2) [22] as is also the case for the Gründlach. Furthermore, in a different study, a strong increase in DTPMP concentrations from 0.5-2 µg/L to 0.7-70 µg/L was reported by Bergdolt et al. after the discharge points of drinking water production sites using reverse osmosis for treatment.[28] Concentrates from this treatment are released directly to surface water and are known to contain antiscalants, often DTPMP.[28]

Regarding the concentration of DTPMP, Jaworska et al. predicted phosphonate concentrations in sediment and surface water calculated using the European Union System for the Evaluation of Substances based on Dutch consumption data from 1998 resulting in an estimated DTPMP concentration of 0.64 µg/L in surface water and 0.1 mg/kg in sediment for local settings in the Netherlands.[23] In comparison, dissolved DTPMP concentrations in the Körsch river, Germany, were between <LOD-0.6 µg/L upstream and 0.3-1.2 µg/L downstream of the WWTP Möhringen,[79] corroborating calculations from Jaworska et al. [23] In contrast, sediment concentrations ranged from 0.05-1.01 mg/kg upstream and 1.21-3.24 mg/kg after WWTP discharge,[79] clearly exceeding values calculated by Jaworska et al.[23] The concentrations in the Gründlach however were clearly higher with 89 mg/kg at the point of discharge.

The strong impact of the WWTP Heroldsberg on the concentrations of aminophosphonates may be due to a specific source such as cosmetics industry present in the city and/or P-elimination rates of 90.9% at the WWTP Heroldsberg [224] compared to e.g. 92.3% for the WWTP Möhringen with the receiving river Körsch [229] with its lower DTPMP concentrations reported by Rott et al.[22]

For DTPMP, a significant decrease in DTPMP sediment concentrations from 89 mg/kg to approximately 10 mg/kg was observed at the discharge point already two months after the closure of the WWTP. This concentration still clearly exceeds the reported concentrations ranging from 0.4-1.9 mg/kg extractable DTPMP in the sediment of the receiving river at the WWTP Möhringen, Germany,[22] but also indicates that the river sediments may recover. To our knowledge, transformation processes in sediment have not been addressed in studies. Possible transformation processes include the photolysis of magnesium, calcium or iron complexes [196] and oxidation in the presence of Mn(II) and oxygen or manganese dioxide.[197, 198] In Addition, downstream transport of sediment particles or mixing of the sediment bed with uncontaminated sediment particles from upstream are likely.

5.4.2 Longitudinal concentration profiles

During operation of the WWTP Heroldsberg, the concentrations of glyphosate and AMPA in water decreased with increasing distance from the WWTP (see Fig. 21A) from 4.5 µg/L directly at the point of discharge to 1.3 µg/L at the sampling site S4, 7.5 km downstream of the WWTP. The trend for AMPA was less pronounced (see Fig. 21A) and concentrations remained high throughout the investigated river reach (15.9 µg/L at the discharge point vs. 13.3 µg/L at 7.5 km). We compared the concentration changes with changes in the electrical conductivity (EC) of the river surface water as a measure for dilution (see Fig. 21A). Glyphosate concentrations largely followed the trend of EC, but at increasing distance to the WWTP the concentrations were higher compared to the trends in EC. In contrast, the higher remaining concentrations of AMPA along the longitudinal profile indicate that processes other than dilution are effective. The lower slope of AMPA concentrations compared to the EC, may indicate that AMPA forms from DTPMP and glyphosate and/or is involved in complex sorption-desorption equilibria with the sediment phase. Input from other sources along the river is possible, however the impact from a tributary creek is expected to be low, as sediment concentration of AMPA was shown to be low with 0.3 mg/kg and no glyphosate was detected in the sediment.

Furthermore, a strong increase by a factor of 24 in AMPA concentrations in the sediment of the Gründlach upon the WWTP discharge (see Fig. 21B) was observed. As for the dissolved concentrations, AMPA concentrations in sediment did not decrease to the same extent as for glyphosate along the river stretch: for AMPA the concentration decreased only by a factor of 2 (from 11.8 mg/kg at the point of discharge to 6 mg/kg approximately 7.5 km downstream), compared to the decrease by a factor of 4 for glyphosate (from 0.8 mg/kg to 0.2 mg/kg) (see Fig. 21B). Comparing AMPA/glyphosate concentration (A/G) ratios in activated sludge supernatant, river water and sediment along the investigated river stretch demonstrated that the A/G ratio from activated sludge supernatant and water directly at the discharge point were in the same scale with values of 3 (WWTP) and 4 (point of discharge, S1). The A/G ratio in water then increased from 8 (2.91 km, S2) to 10 (7.48 km, S4). The same trend can be observed for sediment samples with an A/G ratio of 15 at the discharge point vs. 32 at S4, 7.48 km downstream. Both in water and sediment, AMPA concentrations remained clearly higher than glyphosate concentrations, which indicates possible formation of AMPA, rather than transport of AMPA sorbed to particles or deposition and accumulation of AMPA [230] and subsequent desorption as this would also affect glyphosate concentrations.

For DTPMP, as shown in Fig. 21C, the dependence of concentration on the distance to the WWTP was less distinct compared to glyphosate and AMPA. The highest DTPMP concentration, 89 mg/kg extractable DTPMP, was measured at the discharge point on November 4th, 2023, which coincided with the ending of the WWTP shutdown phase. DTPMP concentration decreased to 7 and 12 mg/kg (September 27th, 2023; November 4th, 2023) at 7.5 km downstream of the WWTP.

With this strong decrease of the concentrations of DTPMP by a factor of about 10 (89 to 10 mg/kg) in sediment compared to glyphosate and AMPA (factor 4 vs. 2), it is likely that this compound is either attenuated by a very strong sorption or by an additional transformation. Upon this transformation, AMPA but also glyphosate may form. In aqueous model experiments, the formation rates in the presence of manganese dioxide were up to 0.06-0.16 mol% for glyphosate and up to 10.1 mol% AMPA,[17] and somewhat lower in sewage sludge (0.02-0.04 mol% glyphosate and 0.40-1.72 mol% AMPA).[44] Desmet et al. used long-term monitoring data sets for a modelling approach to investigate the contribution of WWTP on the surface water contamination. They could clearly show a relative contribution of 29% for glyphosate and around 12% for AMPA from WWTPs discharging directly into the river Meuse with median concentrations in the effluent of 1.7 µg/L for glyphosate and 3.1 µg/L for AMPA, while the median concentration in the Meuse were 0.13 µg/L upstream vs. 0.08 µg/L downstream of the monitored tributaries and WWTPs,[40] far below the concentrations detected in the Gründlach. Interestingly, the highest AMPA concentrations were detected in a tributary receiving discharge from an industrial WWTP, where phosphonates were assumed to be applied in cooling circles. Average concentrations of 28 µg/L AMPA (up to 130 µg/L) were reported, but glyphosate concentrations of only 0.7 µg/L (up to 3.8 µg/L) were detected in a tributary river of the Meuse.[40] This indicates a strong effect of not only municipal WWTPs treating domestic wastewater but also special sources from industrial applications as supposed for the Gründlach from cosmetics industries.

It is interesting to note that the sediment concentrations of glyphosate and AMPA in the Gründlach were rather high also at larger distance from the WWTP compared to the sediment concentrations directly at the discharge point (see Section 5.4.1). Similar concentration trends of glyphosate concentration at the longitudinal profile of the Gründlach can be observed e.g. along the Seine, France, showing an increase of glyphosate concentration in surface water after passing a WWTP, and then decreasing again until passing the next WWTP.[5]

5.4.3 Recovery of the receiving water regarding phosphonate concentrations upon WWTP closure

To understand the effect of the WWTP shutdown on the glyphosate and AMPA concentrations, water and sediment samples from the Gründlach were taken along the longitudinal profile during the shutdown phase of the WWTP from October 15th-18th, 2023 to May 13th, 2024 (for sampling details see Section 5.3.2). As shown in Fig. 23A and B, at every sampling site along the longitudinal profile, the water concentrations of both glyphosate and AMPA were greatly reduced after the shutdown. Glyphosate concentrations ranged from <LOQ to 0.04 µg/L and AMPA 0.28-2.30 µg/L after 8 months compared to the time of operation (glyphosate: 1.35-4.45 µg/L, AMPA: 13.30-15.91 µg/L). At the sampling site S4 (7.48 km downstream), glyphosate concentrations were reduced by a factor of 44 and AMPA concentrations by a factor of 6 comparing the first and last sampling date (27th September, 2023 vs. 13th May, 2024).

Concentrations upstream to the WWTP did not change significantly after the shutdown, as expected, with no detectable glyphosate and with AMPA concentrations ranging from 0.04-0.4 µg/L with some variation due to dilution differences in discharge. The glyphosate concentration profiles showed a concentration peak on October 18th, 2023, which may be caused by flushing the WWTP basins with groundwater, resulting in the release of residual glyphosate. This effect is not visible at the most distant sampling point, probably due to concentrations close to the LOD. This effect caused by flushing of WWTP basins is corroborated by the concentration trends of the pharmaceutical carbamazepine, frequently used as a wastewater marker in surface water.[231] This trend is exemplarily shown for the sampling point S1 (2.91 km) in Fig. 24 (data provided by J. Schaper), where concentration peaks of carbamazepine, glyphosate and AMPA coincide at the 18th October, 2023 (see Fig. 23A and B). The monitoring of the glyphosate concentrations directly at the discharge point during the operation and immediately after the shutdown shows a decrease in concentrations by a factor of 4.8. On May 13th, 2024 the concentration at this location was below the LOQ of 20 ng/L. Using this value as a reference, the factor would be 223. Regarding the sampling site furthest downstream, concentrations were reduced by a factor of 45. For all sampling locations after November 2023, the glyphosate concentrations were between <LOQ and 0.09 µg/L, thus reaching values in the same range as the concentrations upstream to the WWTP.

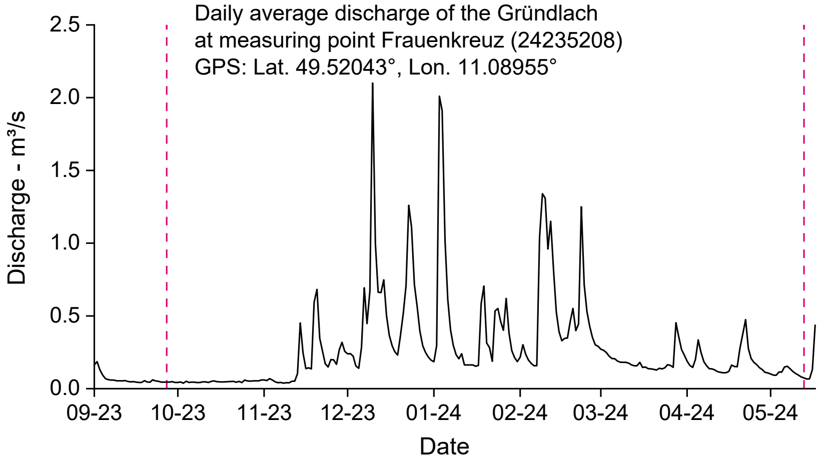


Fig. 22: Daily average discharge of the Gründlach at the measuring point number 24235208 Frauenkreuz (GPS coordinates: Lat. 49.52043°, Lon. 11.08955°), provided by the Gewässerkundlicher Dienst Bayern, Bayerisches Landesamt für Umwelt).[232] Start and end of the sampling campaign are indicated by dashed lines (27th September, 2023 and 13th May, 2024).

The trends for AMPA were slightly different: There was also a strong decrease in AMPA concentrations over the observed time period, but two concentration peaks were visible compared to the glyphosate profile: One peak was observed on October 18th, 2023 which exceeded the initial concentration of those when the WWTP was still in operation, especially at the distance of 4.79 km. Here, the concentration increased from 14.4 µg/L to 18.0 µg/L (see Fig. 23B) and remained at this level at all sites except S4 (7.48 km), where it dropped to 14 µg/L. A second peak in concentration is visible for the sampling points 2.91-7.48 km on November 8th, 2023, reaching a concentration of about 6 µg/L at those sampling sites compared to 0.5 µg/L directly at the former discharge point, which may also be explained by further flushing of WWTP basins with groundwater. Finally, on the last sampling date (13th May, 2024), a slight increase in the AMPA but also glyphosate concentrations was observed at all sampling sites, which can be explained by a drier period resulting in a lower discharge compared to rainfall events from November to February (Deutscher Wetterdienst, location Nürnberg) with the corresponding higher discharge of the Gründlach [232] (see Fig. 22). Presumably, this led to a dilution of the analytes during this time. In general, carbamazepine concentration trends during the sampling time (see Fig. 24) matched well with the concentration trends observed for both AMPA and glyphosate (see Fig. 23A and B), as all three analyte concentrations strongly decreased after eight months compared to concentrations during operation of the WWTP.

As shown in Fig. 23C, the most significant reduction in glyphosate concentration in sediment occurred directly at the discharge point, with values decreasing by a factor of 4 from 0.8 mg/kg to 0.2 mg/kg (27th September, 2023 vs. 13th May, 2024). For AMPA, as shown in Fig. 23D, the decrease was even more pronounced, with concentrations decreasing six fold, from 12 to 2 mg/kg over the same period. Upstream to the WWTP, no changes were observed for either analyte: glyphosate remained undetectable, and AMPA concentrations remained low (0.5-0.7 mg/kg). Clearly, after eight months, the sediments in the river Gründlach had not yet fully recovered from the WWPT impact with regard to these aminophosphonates. A similar trend was observed at the sampling location 2.91 km downstream for glyphosate, but not for AMPA with a reduction by a factor of 4 instead of

6 at this sampling site. However, the decreasing trend was less pronounced at the two most distant sampling sites, where concentrations of 0.07-0.10 mg/kg glyphosate approached the LOQ on 7th February, 2024 and thus were reduced by a factor of 3 for S3 (4.79 km) and of 2 for S4 (7.48 km) (27th September, 2023 vs. 7th February, 2024). An interesting observation was made for AMPA in sediment samples at the most distant sampling points. Concentrations initially increased but then decreased, although less pronounced than for glyphosate. Sampling on October 16th, 2023 showed an unexpected strong short-term decrease for unknown reasons. Remobilization of AMPA due to higher discharge in combination with its longer half-life than glyphosate in soils (12 vs. 3.3 days) [39] and water (52.5 vs. 3.6 days) [40] may explain the late increase at sampling points further downstream of the discharge point.

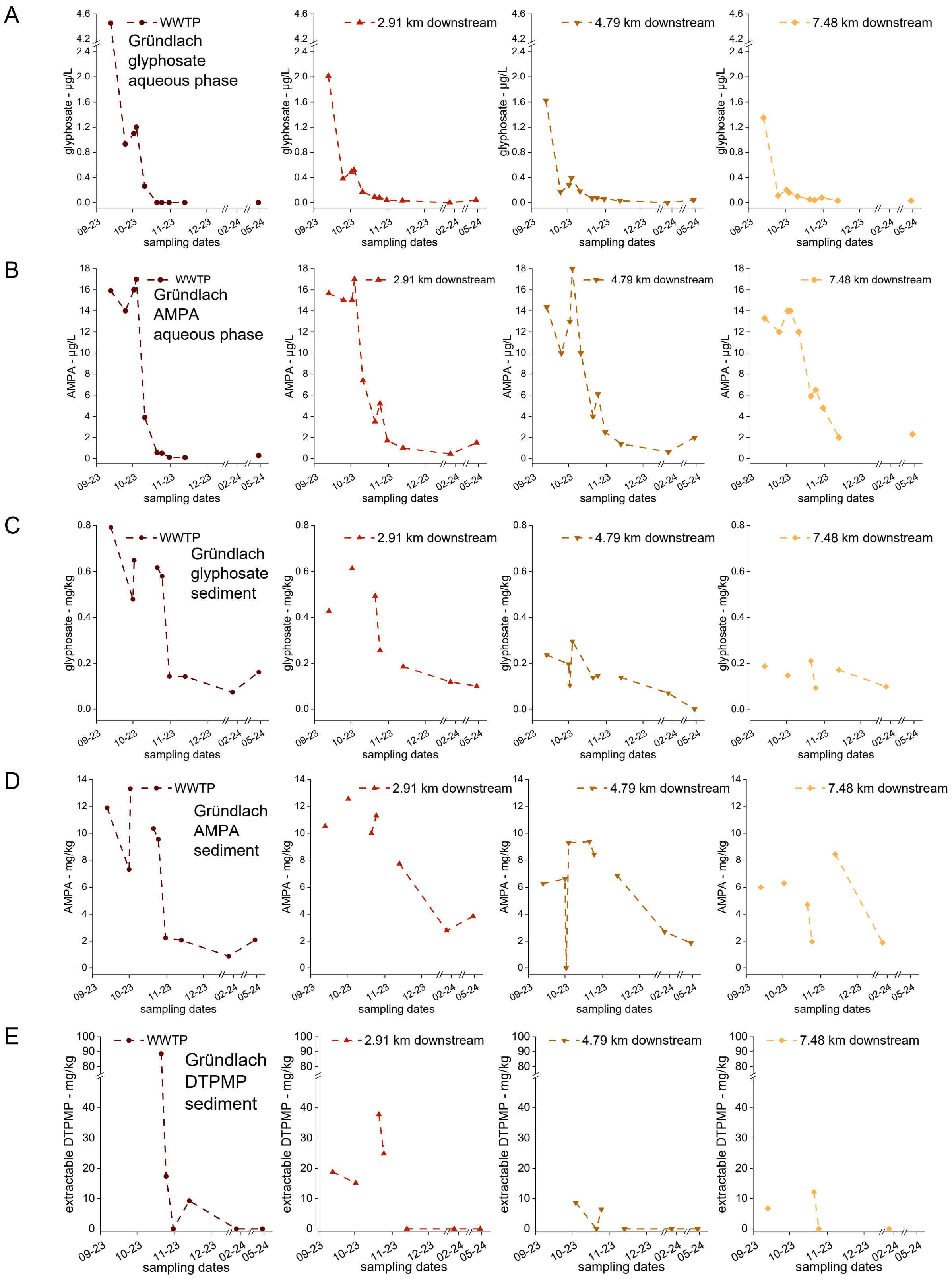


Fig. 23: Longitudinal profiles of (A) glyphosate and (B) AMPA concentrations in µg/L in the water of the receiving river Gründlach and (C) glyphosate, (D) AMPA and (E) DTPMP concentrations in mg/kg in the upper sediment layer (particle size ≤63 µm) along the receiving river Gründlach. Distance from WWTP Heroldsberg: directly at the point of discharge into the Gründlach, 2.9 km, 4.78 km and 7.5 km downstream, see Fig. 20. Sampling dates: September 27th, 2023 during operation of the WWTP, October 15th, 16th, 18th, 2023 during shutdown of the WWTP and November 4th, 8th, 14th, 27th, 2023, February 7th, 2024, May 13th, 2024 after shutdown. Missing sampling dates are due to lack of sample available.

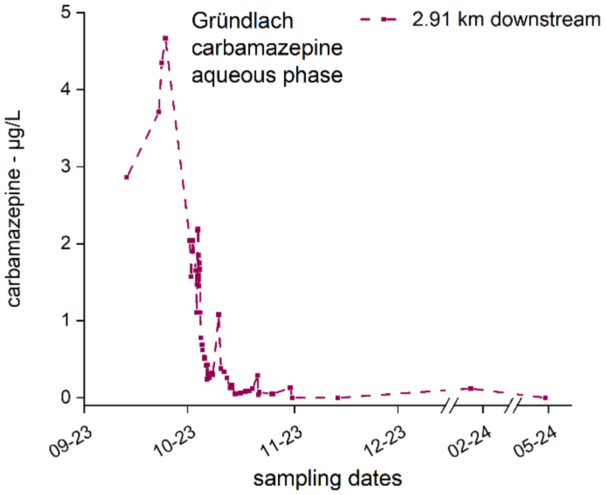


Fig. 24: Carbamazepine concentrations in µg/L in the water of the receiving river Gründlach 2.91 km downstream of the WWTP discharge point (S1) starting on the 27th February, 2023 until the 13th May, 2024. Sampling dates: September 27th, 2023 during operation of the WWTP, October 15th, 16th, 18th, 2023 during shutdown of the WWTP and after November 4th, 2023 after WWTP shutdown. Data kindly provided by J. Schaper.

The counter play of the attenuation processes sorption, transport, transformation/formation of AMPA is complex and the dominant process cannot be clearly identified at present. Full transformation or transport are unlikely as indicated by the analysis of sediment cores detecting glyphosate and AMPA [73] or DTPMP [22] at elevated concentrations down to 20 or 66 cm depth, respectively (see introduction). This demonstrates an accumulation of the original substances and their transformation products, but also transport (possibly, particle-facilitated) as the WWTP discharging into the Anlagensee is about 5 km upstream.

Even though the concentrations strongly decreased over eight months, they would likely not yet meet the environmental quality standards (EQS) proposed by the European Scientific Committee on Health and Environmental Risk for glyphosate of a EQS of 0.1 µg/L (annual average) for surface waters used for drinking water production.[233] All downstream sampling points still exceeded 0.1 µg/L on May 13th, 2024. In contrast, the EQS for sediment of freshwater of 3.08 mg/kg (dry weight) would have been met also with the WWTP being active.

5.5 Conclusion

This study highlights the impact of effluent from the WWTP Heroldsberg on the concentrations of glyphosate, AMPA and DTPMP in sediment and water of the receiving river Gründlach. During the operation of the WWTP, concentrations strongly rose upon WWTP discharge (see Fig. 21), with glyphosate reaching very high concentrations of 0.8 mg/kg in sediment and 4.5 µg/L in water, and AMPA concentrations peaking at 12 mg/kg in sediment and 15.9 µg/L in water. The observed concentrations clearly exceeded concentrations reported in similar studies,[6, 13, 14, 73, 84, 94, 228] indicating the WWTP to be the dominant source of these chemicals likely with a special source in the catchment. These high concentrations of AMPA and glyphosate were paralleled by high concentrations of DTPMP in sediment of 89 mg/kg at the discharge point, which were the highest concentrations in sediments of all studies that reported sediment concentrations for this APP so far. This finding is important as glyphosate and AMPA were shown to be transformation products of DTPMP,[44] so the data here provide a further link of their concentrations and source.

Longitudinal profiles indicate the attenuation of the compounds along the river stretch. For glyphosate, dilution seemed to be a major process, for AMPA, comparisons with conductivity data showed that its formation from glyphosate and presumably also DTPMP or other APPs is likely.

A sharp decrease in glyphosate and AMPA concentrations was observed following the closure of the WWTP, corroborated by the concentration profiles of the wastewater marker carbamazepine over time. For glyphosate, after eight months, concentrations were similar to those upstream of the WWTP. However, the persistence of AMPA indicated by elevated concentration levels in water even months after the closure of the WWTP, suggests that other factors such as formation processes and a relatively long half-life contribute to its environmental persistence. Sediment concentrations of all aminophosphonates decreased but over a longer timespan than water concentrations as can be expected for pollutants strongly sorbing to sediment particles.

We can presume that both sediment and surface water concentrations in the river will further decrease over time. Yet, the different attenuation processes are difficult to differentiate. As discussed, different attenuation processes (sorption/desorption, transformation/formation, transport, dilution) are possible and modelling is required to better understand their relevance. The possible sediment transport of adsorbed DTPMP and its transformation into a variety of transformation products such as glyphosate and AMPA [42, 44, 196-198] and the likely presence of other, large transformation products of DTPMP (e.g. IDMP was detected in one sample, see Section 3.4.2, Fig. 13), further complicates the interpretation of the time profiles of DTPMP concentrations in sediment (see Fig. 23E). In general, however, the decrease in concentrations after the shutdown of the WWTP indicates, that even in a river highly polluted with the aminophosphonates, concentration levels may recover within a year when the source is switched off. However, the reason behind the high concentrations of the aminophosphonates in the WWTP and its receiving river remain unclear. A special source may be present from a large facility of a cosmetic industry present in the city and the known use of phosphonates, e.g. as stabilizers in cosmetics, but other sources are also possible given the broad use of APPs.

Overall, the results emphasize the need for a more comprehensive understanding of the sources and environmental fates of phosphonates and their transformation products. Without a regulation of sources of both the herbicide glyphosate and parent APPs, the proposed EQS for glyphosate in surface waters used for drinking water production will be difficult to reach for rivers impacted by WWTP effluent. And indeed, for glyphosate 92% of member states are expected to exceed the environmental quality standard by even >30% as derived from current monitoring data.[234] Despite the strong reductions in aquatic concentrations observed after the WWTP shutdown, the persistence of especially AMPA contamination in water and sediment at levels exceeding regulatory limits for drinking water production [235] stresses the importance of a specific monitoring and strengthening of mitigation efforts to reduce environmental exposure to these substances. While the main focus was on glyphosate, information on the toxicity of AMPA is still evolving [236] with some studies already indicating negative effects on e.g. amphibian embryos. Further investigations regarding the impact of WWTP shutdowns or the introduction of new treatment stages such as ozonation on the pollution of receiving rivers is crucial to obtain a clear vision on the possible success of mitigation strategies.

6 Summary and Outlook

This thesis clearly contributed to a better understanding of the fate and occurrence of the aminopolyphosphonate (APP) diethylenetriaminepenta(methylene-phosphonic acid) (DTPMP) and its transformation to smaller phosphonates such as glyphosate and aminomethyl phosphonic acid (AMPA) in environmental samples. To facilitate the direct analysis of glyphosate in surface water samples by capillary-electrophoresis (CE)-MS instead of time-consuming chromatographic analysis with reversed phase liquid chromatography (RPLC)-MS after fluorenylmethyloxycarbonyl chloride (FMOC-Cl)-derivatization, soil minerals were tested as sorbents to enrich glyphosate. The most promising results were obtained using magnetite as sorbent due to the strong sorption of glyphosate on the mineral with the advantages of sampling the mineral using magnets. Re-extraction of glyphosate had to be optimized given its strong sorption. An extraction medium combining phosphate, ascorbic and oxalic acid resulted in highest enrichment by a factor of 5, starting with a liquid:solid ratio (water:mineral) of 20 using a spiked water sample. This corresponded to a glyphosate recovery of 66.3%. The success of this extraction medium can be explained by the competition of glyphosate and phosphate for sorption sites [73] and etching of the mineral surface by ascorbic and oxalic acid.[152, 161] This can strongly be improved by increasing the liquid:solid ratio during enrichment, when using high amounts of water samples and low amounts of magnetite for sorption. In addition, the magnetic property of magnetite can ease the enrichment procedure by omitting a drying step prior to extraction by using magnets to collect the mineral after sorption. Due to the strong sorption of glyphosate on magnetite, matrix effects of surface water are not to be expected but nevertheless need to be tested.

Enrichment techniques will ideally be combined with the broadening of analytical techniques to quantify larger APPs such as DTPMP and ethylenediamine tetra(methylene phosphonic acid) (EDTMP) together with their smaller transformation products preferably within one method and without using derivatization. This will help to understand the co-occurrence of APPs and their transformation products such as iminodi (methylphosphonic acid) (IDMP) and glyphosate and enable their monitoring. However, neither the use of a negatively charged coating nor background electrolyte (BGE) modifications such as the addition of organic modifiers enabled detection at of EDTMP and DTPMP at lower concentrations by CE-MS. Still, further work using other BGE modifications such as the addition of adenosinetriphosphate as described by Bernabé-Zafón [67] to reduce analyte-capillary wall interactions is needed. This could aid to inhibit formation of hydrogen bonds between phosphonate analytes and the capillary surface by implementing a phosphate surface where low to negligible sorption can be expected. An alternative is the use of a dynamic coating using phenylphosphonic acid.[191] Nevertheless, MS compatibility has to be considered.

Using more common chromatographic techniques, separations using different hydrophilic interaction liquid chromatography (HILIC) stationary phases were tested for analysis of both DTPMP and EDTMP and a variety of their transformation products. Promising results were obtained using the Accucore 150 Amid column for separation. While separation efficiencies for EDTMP, DTPMP and IDMP were still low due to their higher number of phosphonate groups, the separation of transformation products seems straight-forward. Further optimization is envisaged given that so far, only formic acid was tested as an eluent modifier. Since the eluent pH and ionic strength play an important role in HILIC separation, there is still a great number of possibilities to improve the separation of the phosphonate analytes. As both CE-MS and HILIC-MS were currently not able to detect DTPMP in environmental samples, a derivatization method by Wang et al. [65] was successfully adapted and combined with an extraction method published by Armbruster et al.[52] This allowed us to detect DTPMP using RP-LC-MS in sediment extracts of the same receiving river as the aforementioned transformation products by CE-MS, demonstrating their co-occurrence, all at higher concentrations than in previous studies.

While the formation of glyphosate from DTPMP in aqueous standard solutions in the presence of MnO_2 was already described,[17] we demonstrated the in-situ formation of glyphosate from DTPMP in activated sludge for the first time. This provides further evidence for the important role of wastewater treatment plants (WWTPs) as a source of glyphosate in surface waters. We demonstrated the formation of glyphosate and AMPA in DTPMP incubations using fresh activated sludge and when following the official OECD 302B guideline. Formation of ^{13}C-labeled glyphosate and ^{13}C-labeled AMPA from $^{13}C_5$-labeled DTPMP was demonstrated. The addition of MnO_x to incubation experiments resulted in glyphosate concentrations elevated by a factor of 1.8-3.1 in biotic samples. Together with higher concentrations observed in abiotic incubations, this indicates the predominance of abiotic transformation pathways, likely involving MnO_x. The in-situ formation demonstrated in this study may explain negative elimination rates reported [14] and the reason for the almost ubiquitous detection of glyphosate in WWTP effluent and its rather constant input throughout the year.[5] Nevertheless, it is necessary to further investigate the transformation pathways in aqueous solutions in detail to better understand observations in environmental samples and to identify transformation products to include them in future monitoring studies. Also, formation rates in flow-through experiments simulating the WWTP with its constant influx of DTPMP from e.g. laundry applications and the constant addition of Mn via precipitation agents may aid to obtain more realistic formation rates.

The dominant relevance of WWTPs is further corroborated as this thesis demonstrates the impact of the WWTP Heroldsberg on the concentrations of DTPMP, glyphosate and AMPA in sediment and water of the receiving water Gründlach. Extremely high concentrations of glyphosate (0.8 mg/kg in sediment, 4.5 µg/L in water), AMPA (12 g/kg in sediment, 15.9 µg/L in water) were paralleled by high concentrations of DTPMP (89 g/kg in sediment) directly at the discharge point during operation of the WWTP, while no glyphosate and DTPMP and low AMPA concentrations (0.5 mg/kg in sediment, 0.5 µg/L in water) were detected upstream. A decrease in concentrations of all analytes was observed along the river stretch. The results also demonstrate the output of possible mitigation strategies closing WWTPs as a source of DTPMP and its transformation products: Concentrations of glyphosate and AMPA in water strongly decreased after the WWTP shutdown, proving the emission of the analytes via wastewater, further corroborated by the matching time-profile with the wastewater marker carbamazepine. After eight months, glyphosate concentrations in water were similar to those observed upstream and close to the LOD, while AMPA concentrations were still elevated, suggesting prevailing sources such as the transformation process from phosphonates in sediments. In addition, its longer half-life may be important. Sediment concentrations did not show such a clear trend as water concentrations, probably due to the strong sorption of aminophosphonates. Nevertheless, a decrease in sediment concentration was observed for all analytes. Concerning the sediment and water concentrations of glyphosate, AMPA and DTPMP we can presume a recovery of the river after WWTP closure. Yet, it is not clear which processes dominate regarding the attenuation of analytes along the river stretch. In general, dilution, transformation/formation, sorption/desorption and particle-facilitated transport need to be discussed in further studies. It is clearly necessary to intensively investigate the transformation pathways of APPs, but also the co-occurrence with their transformation products in receiving rivers of WWTP. Monitoring of WWTP shutdowns or commissioning of new treatment steps such as ozonation in WWTP can clearly help to identify sources of pollutant emissions enabling a better regulation or improved elimination. With the new insights gained from the work in this thesis, a new source for glyphosate was discovered. This knowledge may partly explain the constant and unchanged glyphosate loads observed in surface waters over years, even after implementation of strategies to reduce its usage, and now allows us to take new measures to reduce glyphosate and AMPA concentrations in surface waters.

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Appendix

Supplementary Information to Section 2.2

Glyphosate contamination in European rivers not from herbicide application?

In this supporting information we provide background information on international glyphosate application data and its agricultural use in Europe. We discuss that urban herbicide applications cannot be considered the main source for glyphosate in European wastewater treatment plants. Finally, recent mitigation strategies in Europe are summarized.

Additional figures show the log(A:G) ratios for selected sites (Fig. S1), an overview on glyphosate and AMPA concentrations for countries with a large number of data sets available in this meta-analysis (USA, France, Germany) (Fig. S2). Fig. S3 adds examples for mass fluxes to Fig. 6 in the main text. In Fig. S4 we plot published data on glyphosate and AMPA findings in dried sewage sludge. Table S1-1 and Table S1-2 provide data for glyphosate, AMPA, discharge, agricultural markers and wastewater markers for sites in the USA and Europe in separate documents. Finally, Table S1-3 and Table S1-4 summarize Spearman rank correlation coefficients between glyphosate and other pesticides or micropollutants entering rivers via wastewater.

S1-1 Glyphosate application data for the USA and European countries

International glyphosate use in tons (in brackets: tons per 100,000 capita, t/10^5 c) was:

USA: 125,000 tons (38 t/10^5 c) in 2014 [237]

France 9,300 tons (14 t/10^5 c) in 2017 [238]

Sweden: 485 tons (5 t/10^5 c) in 2017 [238]

Germany: 4,700 tons (6 t/10^5 c) in 2017 [238]

the Netherlands: 742 tons (4 t/10^5 c) in 2017 [238]

Italy: 3,700 tons (6 t/10^5 c) in 2017 [238]

United Kingdom 2,240 tons (3 t/10^5 c) in 2017 [238]

Luxemburg: 23 tons (3 t/ c) in 2016 [238]

This gives rise to a glyphosate application rate in terms of total agricultural area of: 138 kg/km^2 in the USA,[115] and for the above-mentioned European countries 26 kg/km^2 on average (ranging from 17 kg/km^2 for Luxemburg and the UK to 32 kg/km^2 for France).[116]

S1-2 Agricultural use of glyphosate

Clearly, the contamination patterns in Europe with their strong seasonality do not fit to agricultural applications as exemplified for Germany: In a large survey from 2017, it was stated that about 37% of agricultural land was treated with glyphosate, 60% of glyphosate was used for stubble applications (ca. 22% of agricultural land), 34% pre-sowing applications (13% of agricultural land) and 6% for pre-harvest applications ("siccation") (2% of agricultural land).[3] Other studies reveal similar percentages.[114, 239-241] In Germany in 2014/15, the percentage of agricultural area of a crop treated with glyphosate were: 72% in winter rapeseed >48% sugar beet root >33% winter barley >31% winter wheat >27% maize >26% leguminosae.[241] Berger et al. showed glyphosate applications in March and May [242] (for direct sowing in maize),[114] and siccation applications in June/July, but the dominant application of glyphosate is for stubble cultivation in August to October. Regulations in Germany from 2014 require a minimum of 90 days between two treatments.[243] This means that either stubble or pre-sowing applications are possible when winter crops are cultivated. An additional pre-sowing application can only be made for a successive crop. Thus, for winter crops, the stubble treatment is often in September, while October is chosen for the pre-sowing applications. Thus, we would expect elevated intense quickflow-induced glyphosate input into rivers in March-May and September-October and lower concentrations during the rest of the year. This is not observed in the data of this meta-analysis for Europe. In contrast, for the USA, elevated concentrations better correlate with times of application (see Section 2.2.4.1 in the main text). Siccation could be expected to be the main cause for elevated glyphosate concentrations in June and July. However, siccation was strongly regulated in recent years (see Section S1-4), leaving applications in summer to special crops. However, no distinct differences for vine-growing regions in France (sites E20, E24, E26, E29-31, E34) are visible compared to sites with a high share of agricultural fields or grassland. Regional differences in land use, crop type, farm size,[244, 245] etc. cannot be retrieved in the data presented in Table S1-2. Instead, similar patterns are present at sites spear all over the countries investigated. Thus, high mass fluxes in early summer are difficult to explain from agricultural herbicide applications.

S1-3 Urban applications of glyphosate and relation to concentration patterns in rivers

Findings of glyphosate in WWTPs have mostly been ascribed to urban applications of the herbicide and subsequent mobilization into the sewer system. In this chapter we discuss different urban sources for the herbicide glyphosate possibly leading to its presence in wastewater.

S1-3.1 Municipal and private applications: Municipal applications, e.g. along streets and applications on industrial or commercial sites, e.g. parking spaces, etc. are frequently discussed to be a major source for glyphosate. Today, these applications need a specific permit, which is hardly granted in Germany anymore: E.g. in Baden-Württemberg, only 89 permits for non-agricultural applications of plant protection products (mainly but not only glyphosate) were issued in 2020 for the whole state.[246] In Berlin, 11-29 permits per year were granted in 2018-2021 (information provided by the Pflanzenschutzamt Berlin). Along with the decreasing numbers of permits, the area allowed to be treated with a maximum of two applications during the growing season (April to September) (Pflanzenschutzamt Berlin) is reduced. Private and municipal applications can be roughly estimated by the sales numbers for non-occupational applications. These declined from 95 tons per year in Germany in 2014 to 9 tons in 2020 and 17 tons in 2021 (statistics from the German Bundesamt für Verbraucherschutz und Lebensmittelsicherheit 2022).

Agricultural loss rates were reported to be <1%.[11, 89, 98] Similar loss rates were described for residential areas estimating glyphosate applications either via controlled applications [90] or via questionnaires.[91] Recoveries of 0.5% in a storm drain outflow [90] and 0.6% in storm water drains [91] were reported. In both cases, surface runoff was the main cause for glyphosate transport into sewers or surface water, with first flush phenomena [247] reported similar to transport in agriculture.[89] Most runoff can be expected from sealed surfaces, e.g. parking lots, where up to 35% of the applied amount was recovered when sampling around the edges of rather small, sealed areas.[92] All herbicides applications described here can be expected to lead to pulses of glyphosate (and AMPA) entering the sewer system or rivers directly during rain events, which is not compatible with European river water patterns and the rather constant base mass fluxes observed (see Fig. 6 and Section S1-3).

S1-3.2 Application from railway tracks: The Deutsche Bahn reported that 90% of the railway tracks were treated once per year with herbicides (78 and 65 tons in 2015 and 2017, respectively),[4] in rare cases more than one application was made. Glyphosate use was strongly reduced in recent years (websites of Deutsche Bahn), but this did not evoke changes in glyphosate contamination in rivers.

Several reports discussed glyphosate losses from railway track applications, e.g. findings in groundwater close to railway tracks with reduced detection frequencies at larger distances.[123] Rain-driven input with concentration peaks can be expected with regard to a study on diuron used at railway tracks before glyphosate was preferred to combat weeds: Skark et al. observed sharp glyphosate peaks after rain events.[248] Surely, applications at railway tracks cannot explain the constant mass fluxes seen all over (Western) Europe and glyphosate's presence in wastewater all over the year.

S1-3.3 Input via diet and urine: The only source known to us which would evoke a rather constant mass flux of glyphosate into WWTPs, is human diet. A median urinary glyphosate concentration (24 h) of 0.1 µg/L for children was reported in a large study in Germany.[249] Calculating with 2 L urine per day for 83.2 million inhabitants in Germany (statistics from 2021), we calculate a urinary input of roughly 6 kg/a in Germany into WWTPs. Considering elimination rates of 80% in WWTPs,[13] only 1.2 kg of glyphosate would enter German surface waters via this pathway. This is clearly lower than the mass fluxes calculated in this and other studies (see Section 2.2.5.2, main text). Even when using the 90% percentile (0.35 µg/L) of the urinary concentrations of the study,[249] loads of only 4.3 kg/a could be explained for Germany. For site E44, with 5.5 million people in the catchment, we calculate <0.1 kg per year using the median urinary concentration, which is low compared to the annual load of 360 kg determined from surface water concentrations and discharge at this site. This estimation shows that an input pathway via urine is not a major cause of the glyphosate concentrations in rivers.

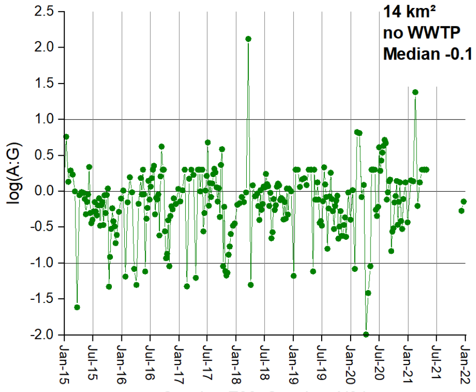
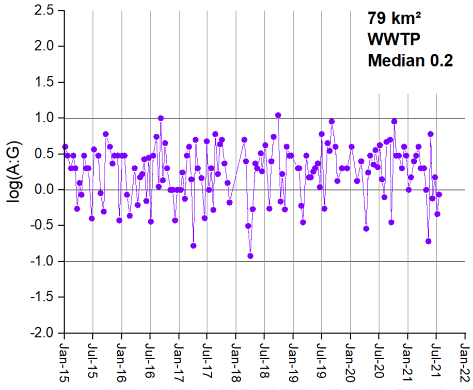
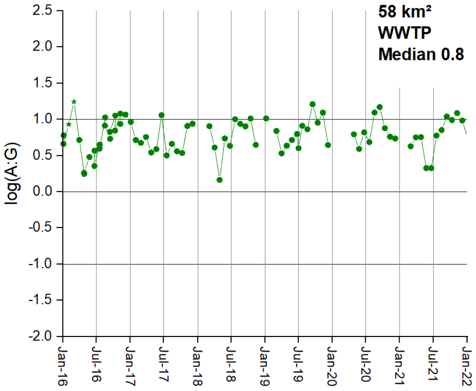
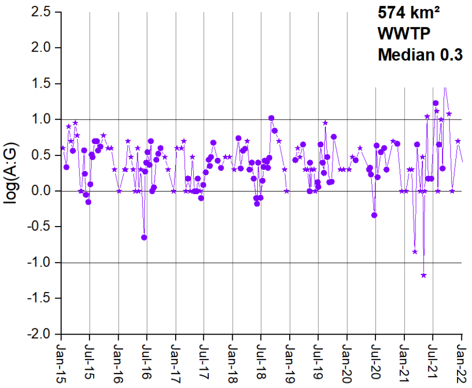
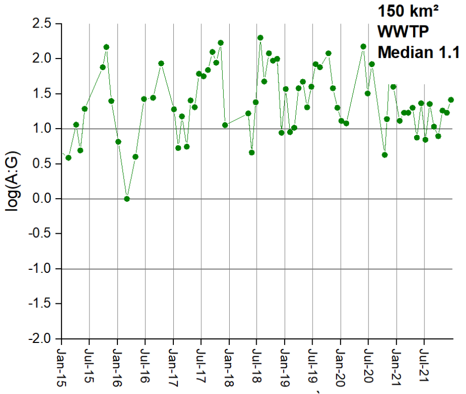
S1-4 Mitigation strategies in Europe

Several mitigation measures were implemented in recent years in Europe. For example, since 2014, German regulations allow a maximum of two applications with at least 90 days between the treatments and a maximum of 3.6 kg active agent per hectare and year.[243] Siccation was first limited to specific applications in small areas, when there was a risk to lose the crop, but since 2014, siccation is strongly restricted in Germany and was fully banned in 2021, when also further severe restrictions for agricultural applications were implemented.[96] At that time, model calculations show that with this reduction of glyphosate application, the leaching and discharge into surface waters could be limited to remain below 0.1 µg/L. [243] However, looking at the surface water data presented in this meta-analysis, these mitigation strategies were not successful to reduce river water concentrations, except eventually for the Neckar and the Main (sites E44 and E55).

In 2015, hardware stores partially stopped selling glyphosate in Germany. Since 2021, the use of glyphosate in house, kitchen and gardens is forbidden in Germany. The EU restricted glyphosate usage in public gardens, playgrounds and parks already in December 2017.[250] Several cities and municipalities voluntarily decided to ban pesticide applications, sometimes even more than 10 years ago, which could have been expected to have reduced surface water concentrations for urban catchments as described in Canada,[251] but this has not or not yet happened.

The strongest mitigation measure for glyphosate was implemented in Luxemburg in 2021 with a complete ban of glyphosate. However, concentration patterns and absolute concentrations remained the same at the sites E70-73, despite the major fraction of their catchment being located in Luxemburg (Alzette ca. 5% from France, Syr ca. 25% from Belgium). For comparison, in Australia, surface water concentration of diuron increased within half a year upon announcements of restrictions, pointing to the use of stocks.[252] When implementing restrictions (here, a no-spray window for diuron), diuron concentrations quickly diminished 4-fold lower. Thus, within the period of a complete ban of glyphosate in Luxemburg, changes in the surface water concentrations could have been expected.

Supplementary figures

U.S. sites	European sites
	 Sweden E39: Sweden_N34
 USA U12: 02335870_SOPE CREEK NEAR MARIETTA, GA	 Luxemburg E73: L300030A06_Chiers - Rodange pont à Athus
 USA U7: 05451210_South Fork Iowa River, New Providence, IA	 France E15: 2106500_LA MEUSE À BASSONCOURT

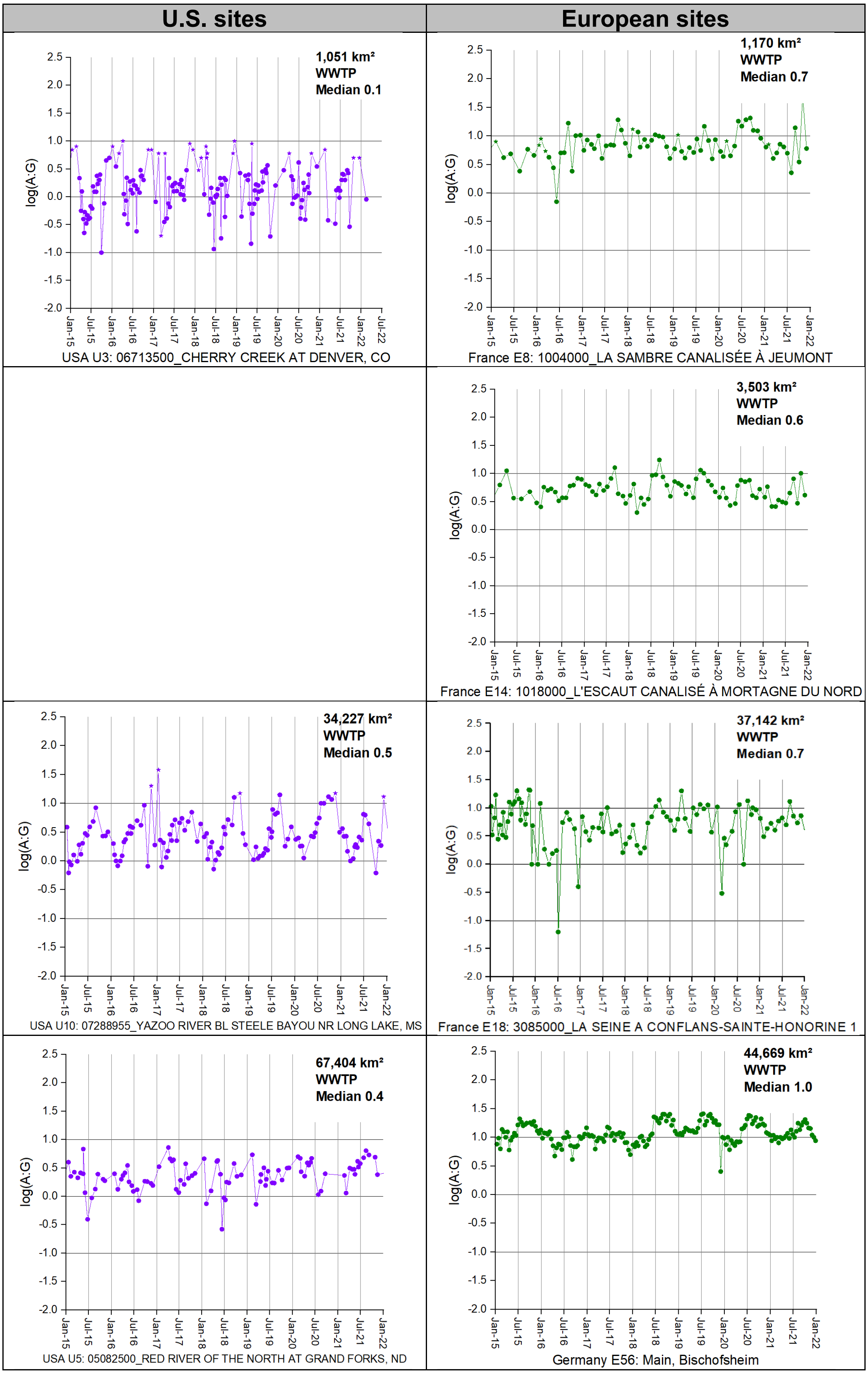


*Fig. S1: Ratios of log(AMPA:glyphosate), log(A:G), for different sites. Size of the catchment, WWTP impact and median log(A:G) given in the panels. Concentration data <LOD were replaced by ½ LOD. U.S. and European sites with a similar catchment size are presented in one row. Data points plotted with an asterisk were <LOD and calculated to 0.5*LOD. Median values were calculated using these data.*

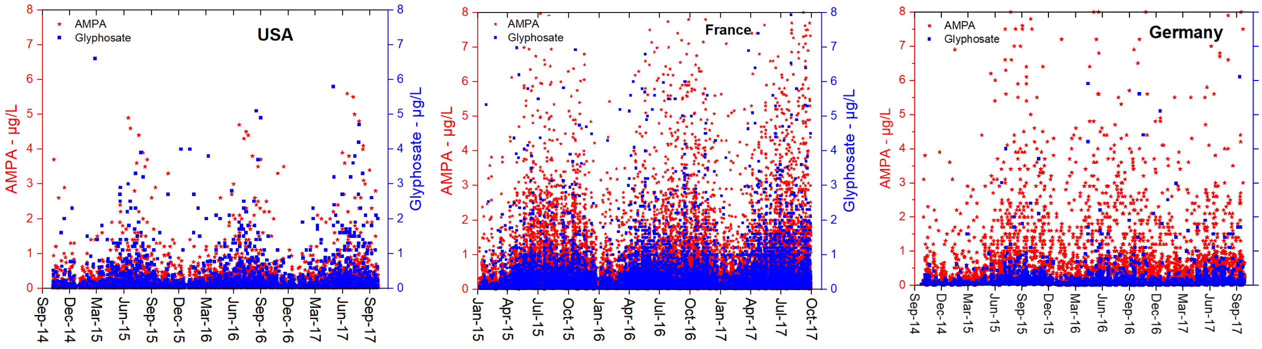
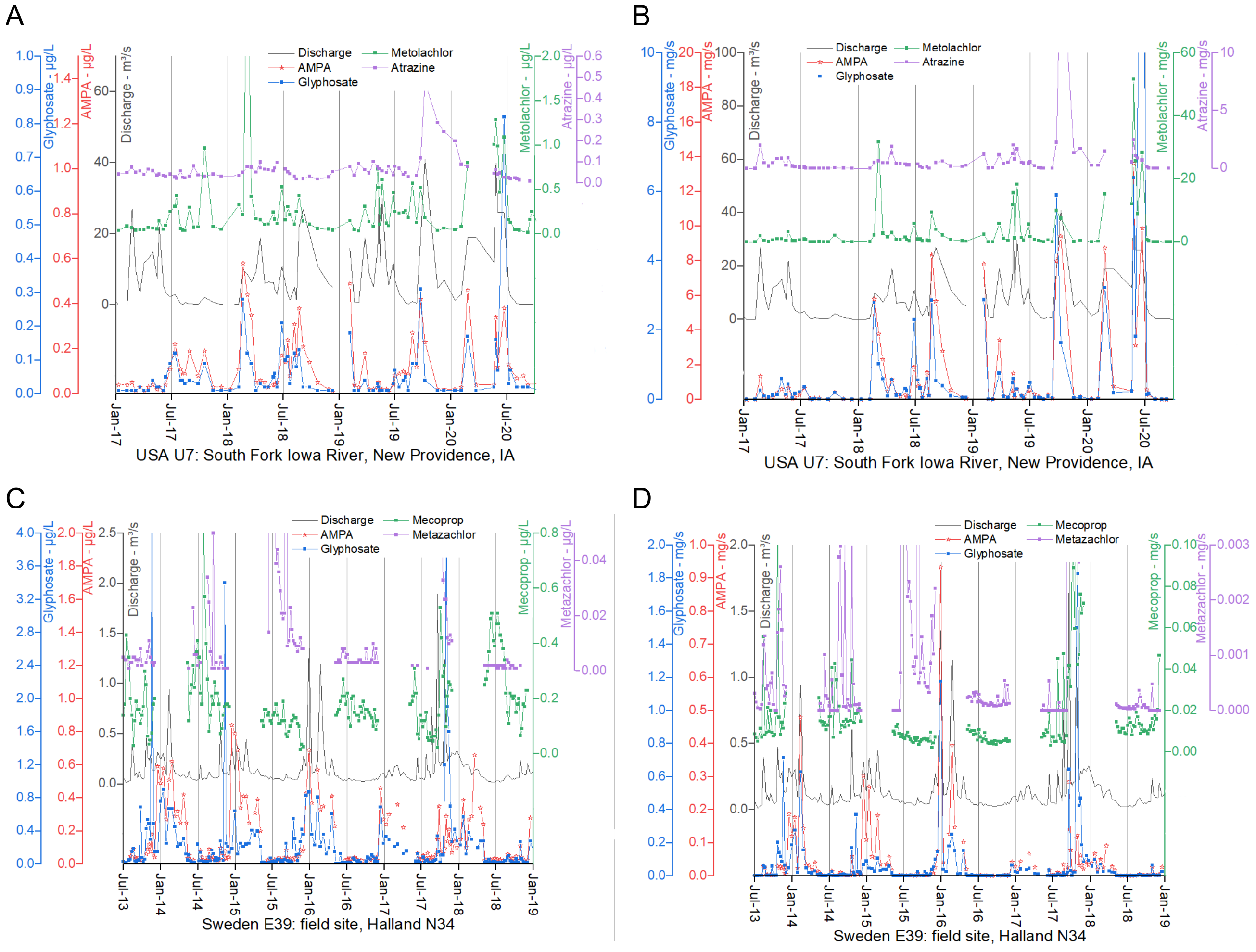


Fig. S2: Overview of glyphosate and AMPA concentrations for the years 2014-2017 (best data availability) for USA (3,200 data points), France (66,900 data points) and Germany (4,200 data points) using concentration data >LOD. Graphs scaled to 8 µg/L (some data points are >8 µg/L). Data sources, see Table S1-1 and Table S1-2. Additional data for the river Ruhr, Germany, kindly provided by the Westfälische Wasser- und Umweltanalytik GmbH.



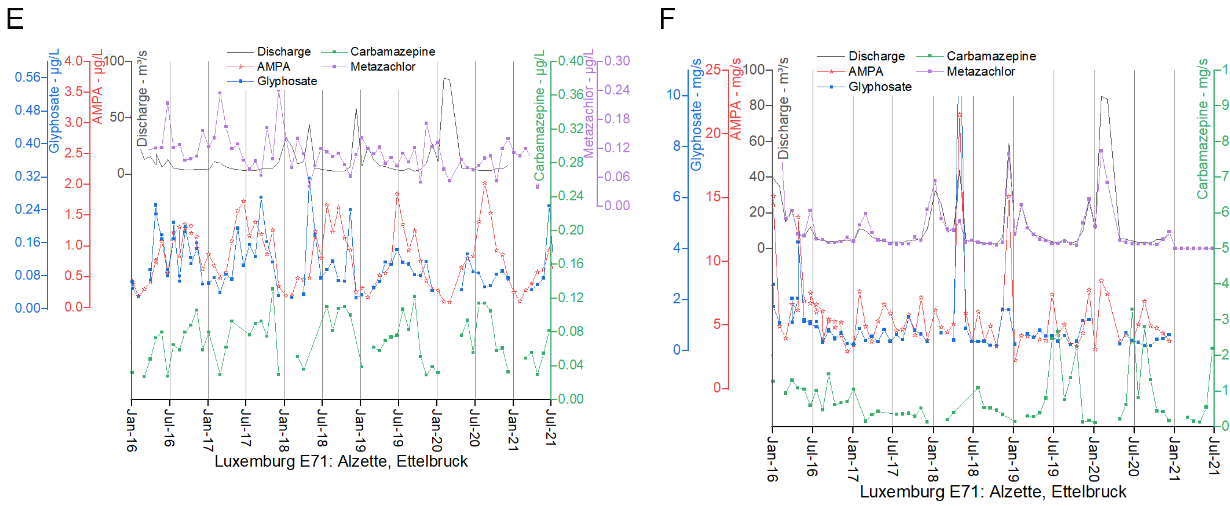


Fig. S3: Comparison of concentrations vs. mass flux time series for selected rivers in the USA (South Fork Iowa River, site U7 with an agricultural catchments), Sweden (site E39 with a purely agricultural catchment), in Luxemburg (site E71 with a mixed catchment). Site details and data sources in Table S1-2. Complement to Fig. 7, main text.

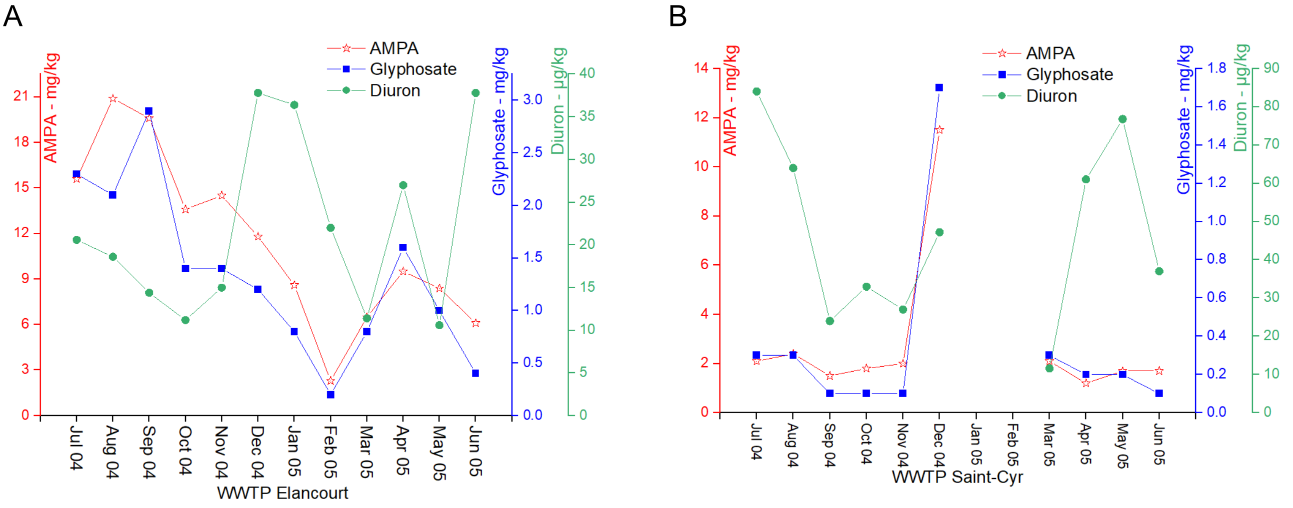
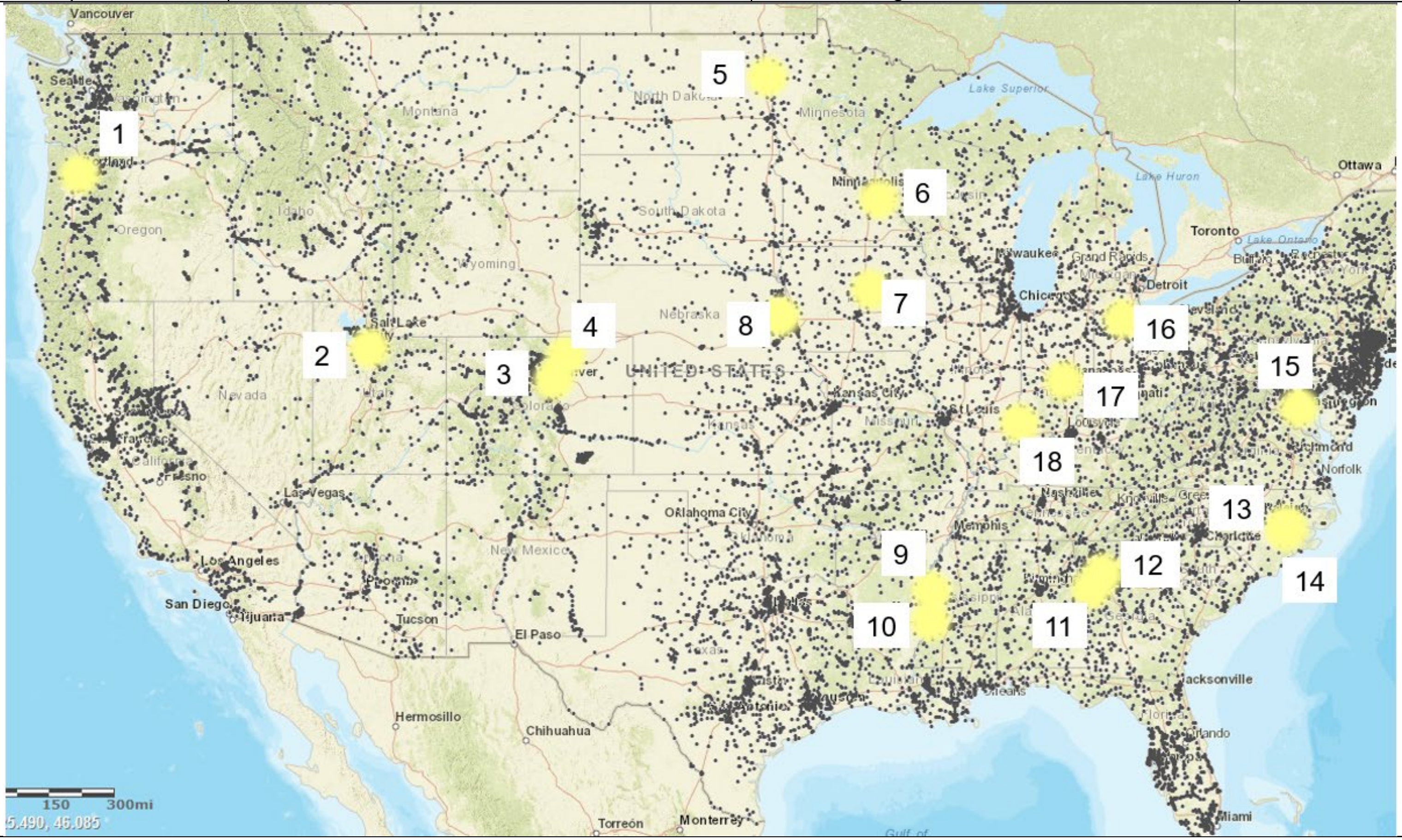
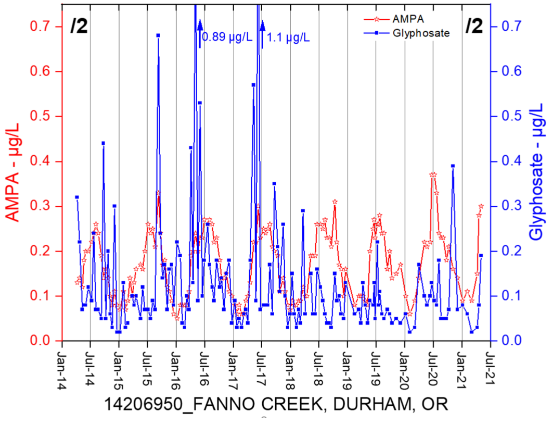
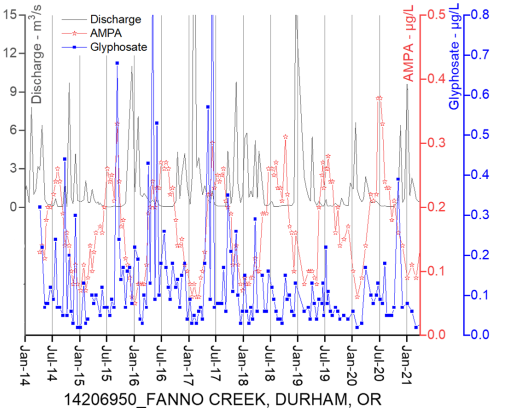
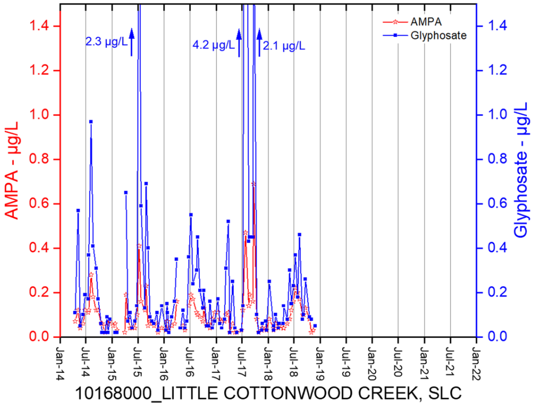
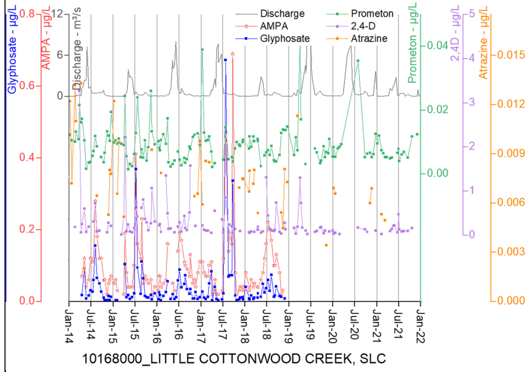


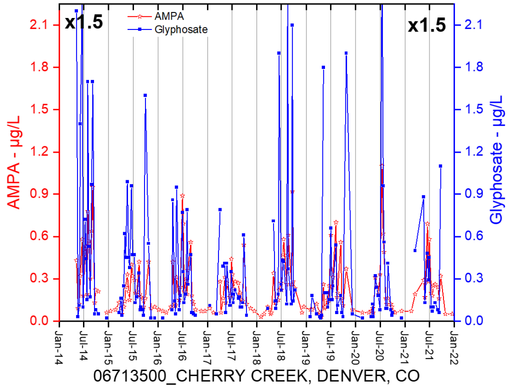
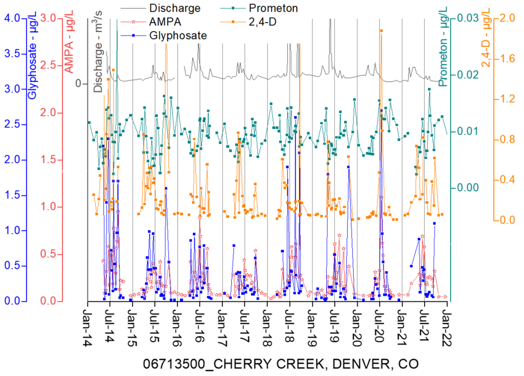
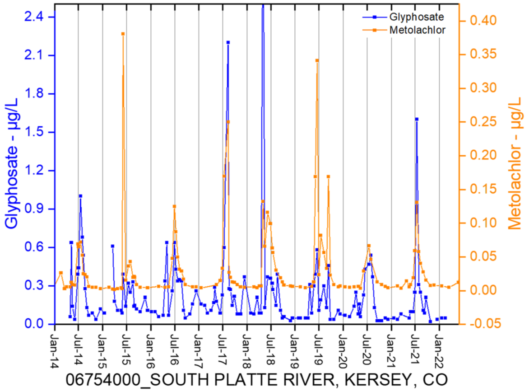
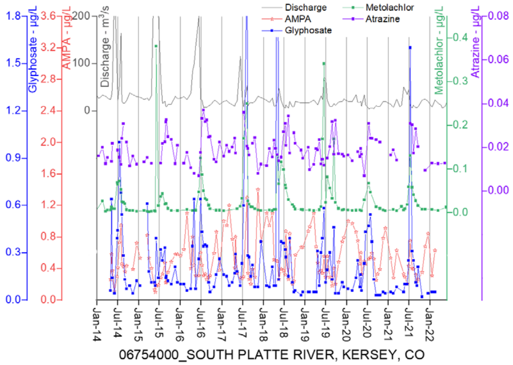
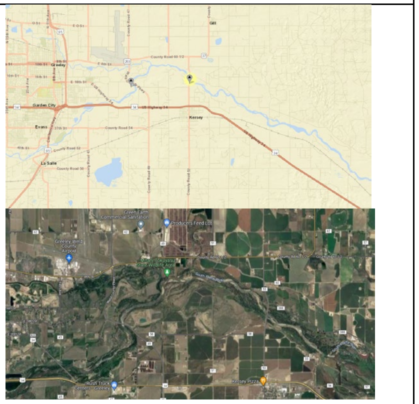
Fig. S4: Glyphosate, AMPA and diuron concentrations in dried sewage sludge in two French WWTP. Data taken from Ghanem et al. for the WWTPs Elancourt (separate sewer system, 42,000 IE) and Saint-Cyr (combined sewer system, 200,000 IE);[87] French inhabitant equivalents taken from https://www.assainissement.developpement-durable.gouv.fr/PortailAC/data in 10/2023 accessed in 2023.

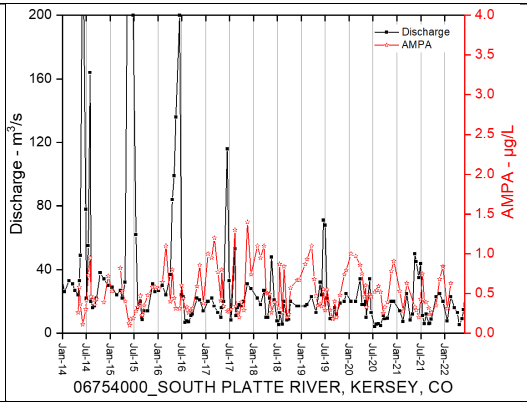
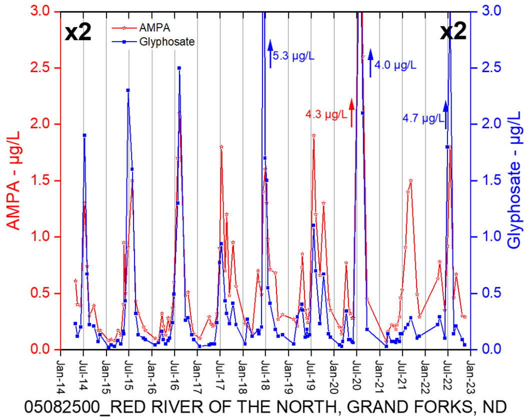
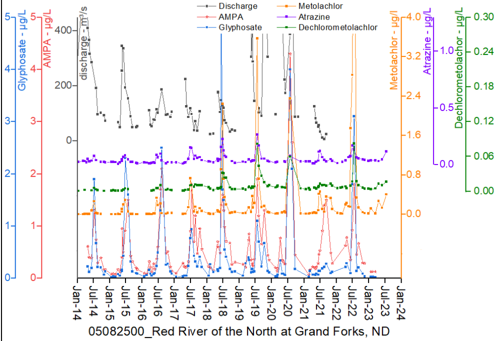
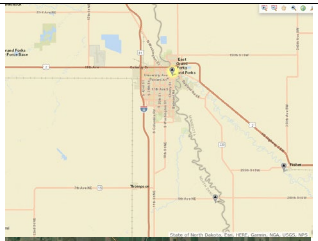
Supplementary tables

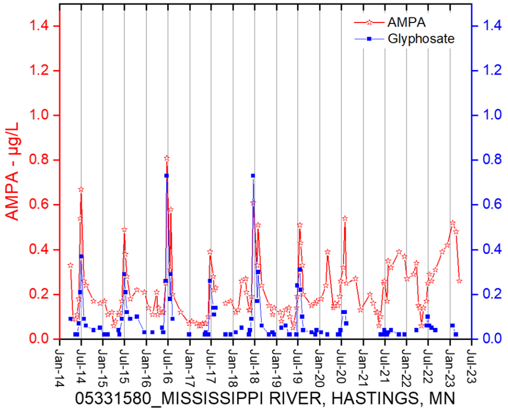
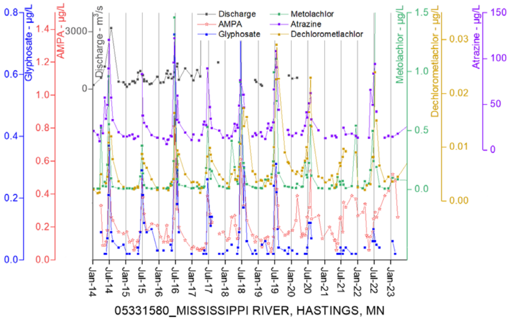
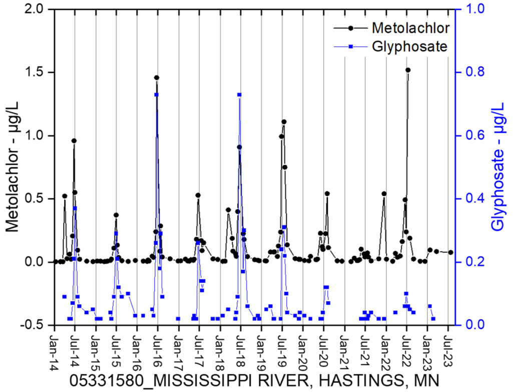
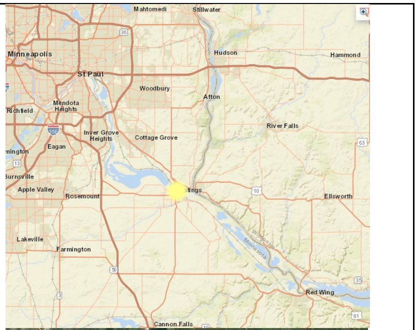
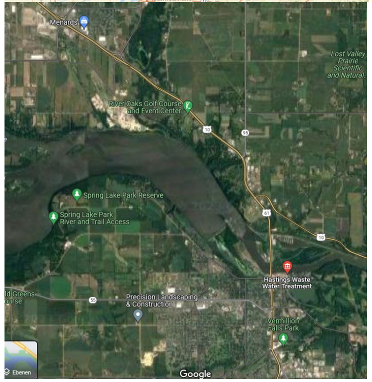
Table S1-1: Overview on sampling sites all over USA. Temporal patterns for U.S. sampling sites for glyphosate and AMPA mostly scaled to 1.5 µg/L for both AMPA and glyphosate (differing scales are indicated with e.g. /2 or x2), and their comparison to discharge and other herbicide concentration patterns. In all plots, glyphosate and AMPA are scaled identically. The site number and name are those used by the USGS. Data and map taken from the National Water Information System: Mapper (https://maps.waterdata.usgs.gov/mapper/index.html are plotted. Googlemaps or maps bing aerial view were used, whichever provided better resolution.

Site	Site name and description	Seasonal patterns for glyphosate and AMPA	Comparison to agricultural micropollutants and discharge	site location and aerial view
				

U1 **OR**	**site:** 14206950_ FANNO CREEK AT DURHAM, OR **catchment:** 81 km² fully urban catchment **WWTP:** no WWTP			
U2 **UT**	**site:** 10168000_LITTLE COTTONWOOD CREEK @ JORDAN RIVER NR SLC **catchment:** 118 km² spring in mountains with forests, flows through Salt Lake City influence of snow melt **WWTP:** no WWTP			

U3 CO	**site:** 06713500_CHERRY CREEK AT DENVER, CO **catchment:** 1,051 km² source in agricultural area; Cherry Creek Reservoir upstream; sampling site in Denver **WWTP:** Parker (ca 60,000 inh.; UV disinfection)			
U4 CO	**site:** 06754000_SOUTH PLATTE RIVER NEAR KERSEY, CO **catchment:** 24,760 km² sampling site downstream Denver **WWTP:** Denver (2.2 Mio IE with chlorination, since 2018 peracetic acid) Denver (since 2016, 100,000 IE, UV disinfection)			

			 	
U5 **ND**	**site:** 05082500_RED RIVER OF THE NORTH AT GRAND FORKS, ND **catchment:** 67,404 km^2 intensive agricultural catchment **WWTP:** Fargo (125,000 inh., UV disinfection)	 	 	 

U6 **MN**	**site:** 05331580_ MISSISSIPPI RIVER BELOW L&D 2 AT HASTINGS, MN **catchment:** 95,083 km² City of Minneapolis and agricultural land upstream; **WWTP:** Minneapolis (1.8 Mio IE, chlorination)		 	 

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IA</td>
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catchment: 574 km²
fields and grassland in catchment, sparsely populated, forest at river shore
WWTP: no WWTP</td>
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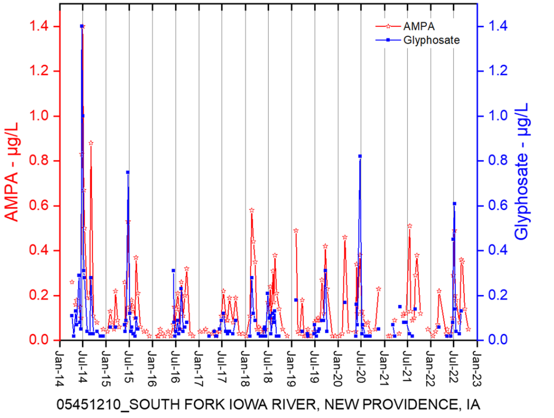

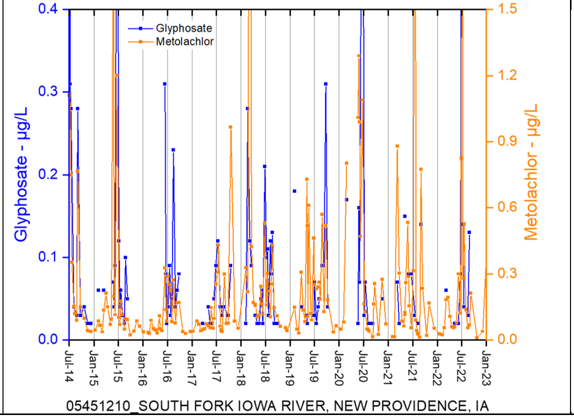

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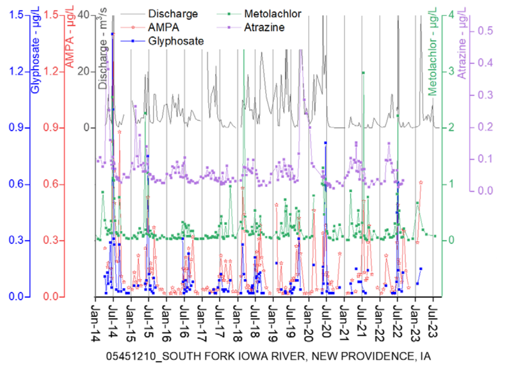

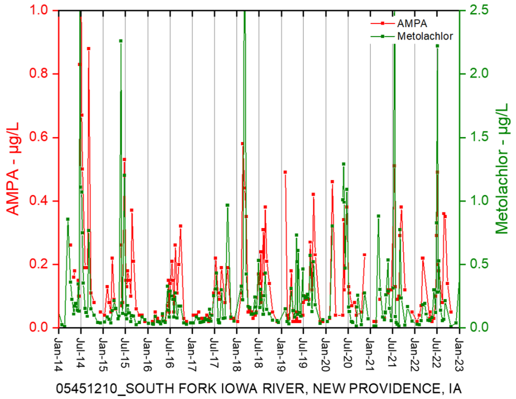

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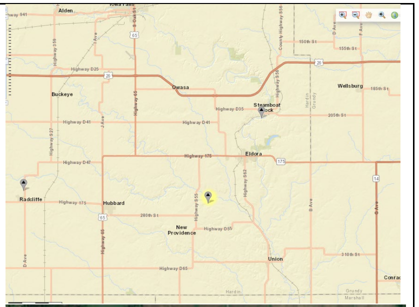

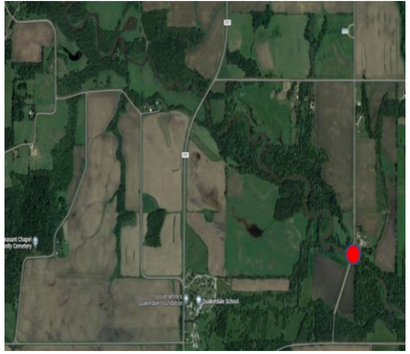
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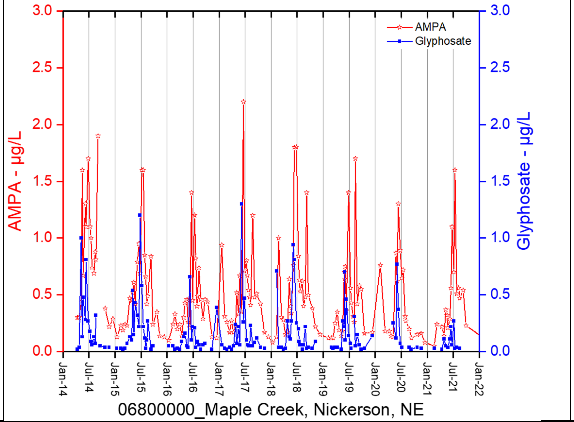
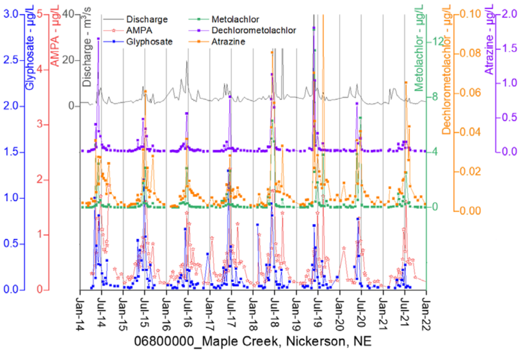
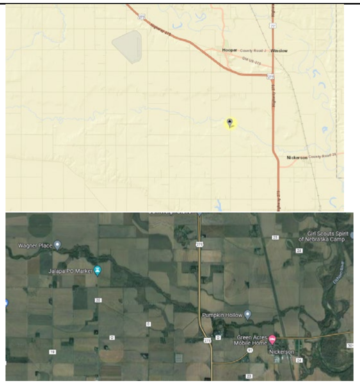
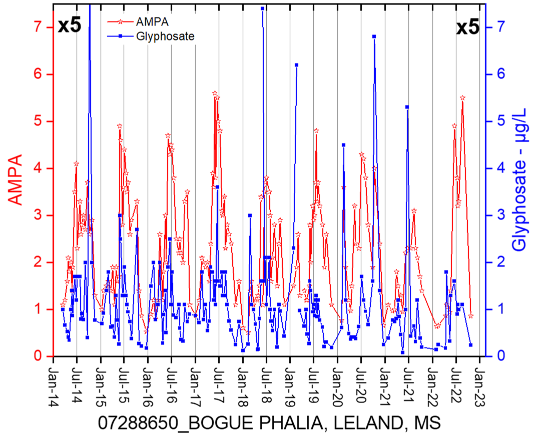
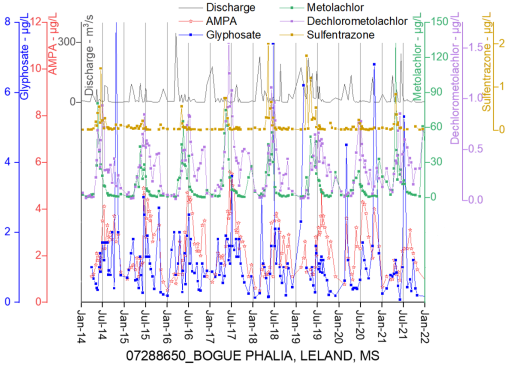
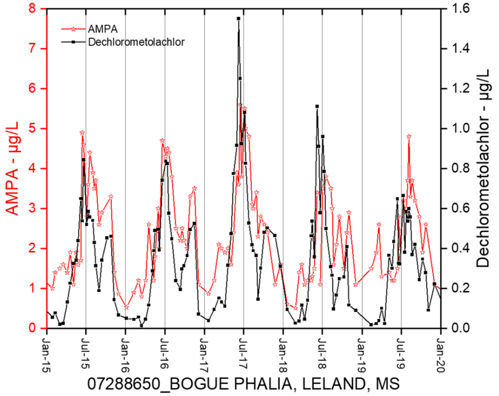
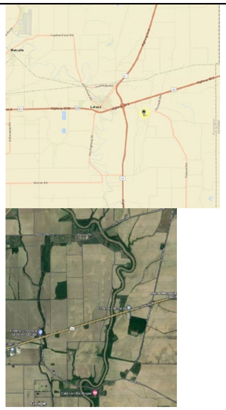
U8 **NE**	**site:** 06800000_Maple Creek near Nickerson, Nebr. **catchment:** 943 km² intense agricultural land use in catchment, partially with irrigation catchment sparsely populated **WWTP:** no WWTP			
U9 **MS**	**site:** 07288650_ BOGUE PHALIA NR LELAND, MS **catchment:** 1,240 km² intense agricultural land use **WWTP:** low WWPT input if any		 	

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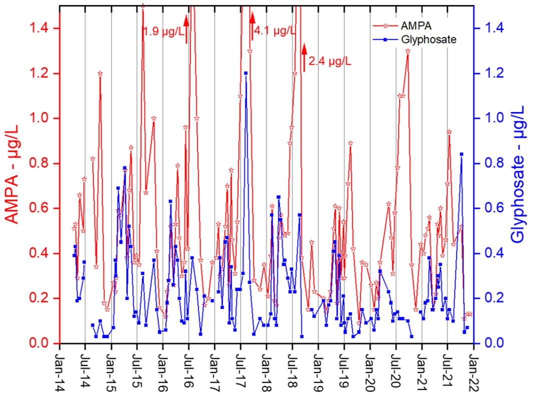
MS</td>
<td>site: 07288955_YAZOO RIVER BL STEELE BAYOU NR LONG LAKE, MS
catchment: 34,227 km²
agricultural land use, sampling site at the estuary to the Mississippi
WWTP: Yazoo City (11,000 inh.), several smaller WWTPs</td>
<td>
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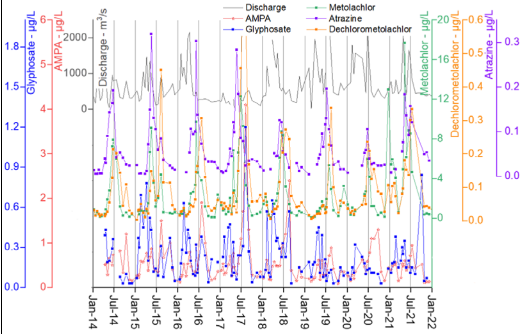
<td>
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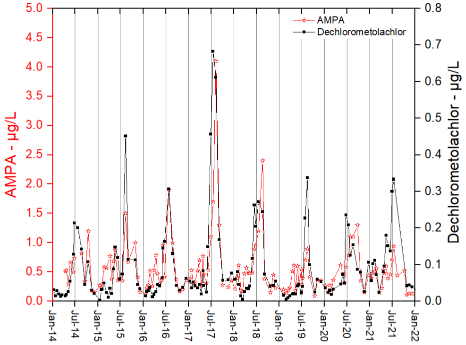
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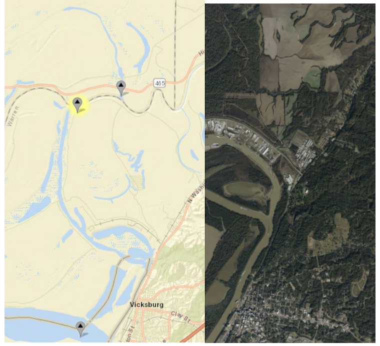
<td> </td>
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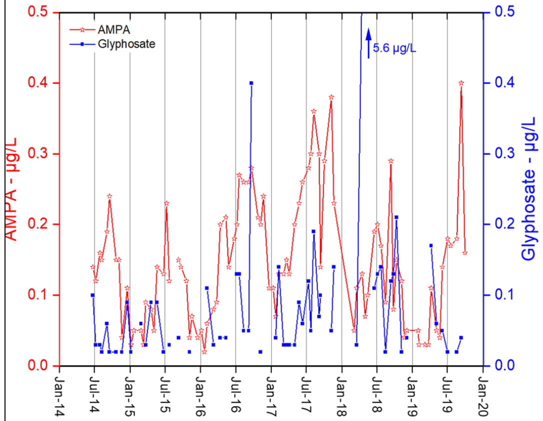
<td>U11
MI</td>
<td>site: 02338000_CHATTAHOOCHEE RIVER NEAR WHITESBURG, GA
catchment: 6,228 km²
fields and forests in the catchment, downstream of Atlanta and suburbs
WWTP: 2 large WWTPs in Atlanta, UV disinfection</td>
<td>
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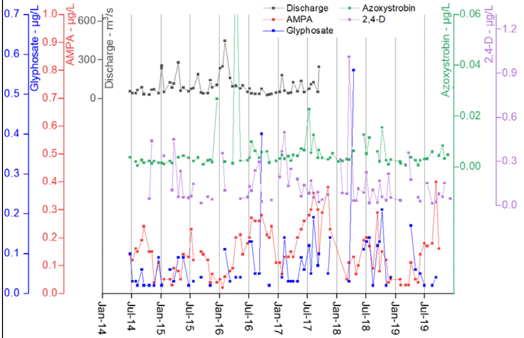
<td>
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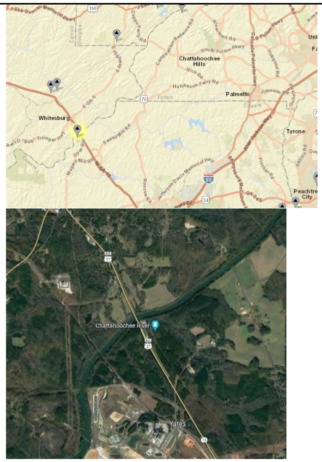
<td> </td>
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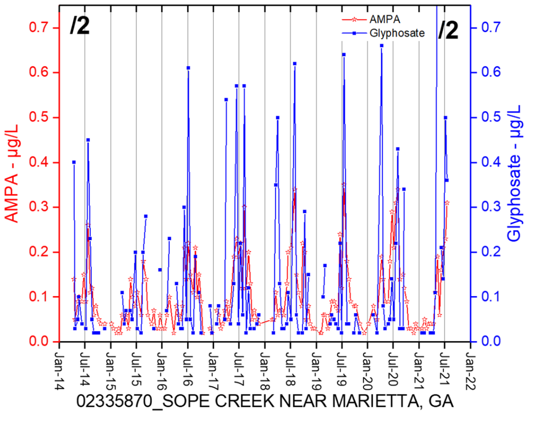
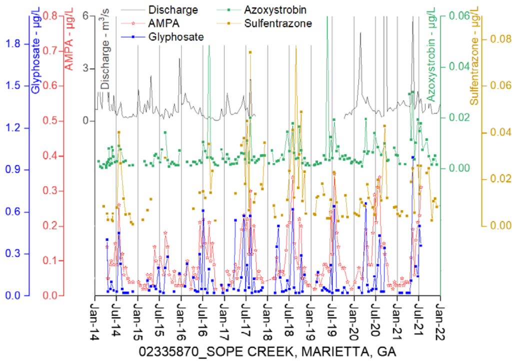
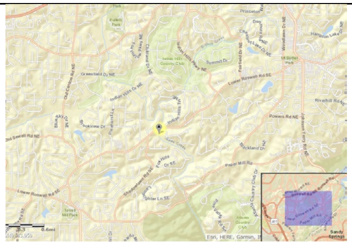
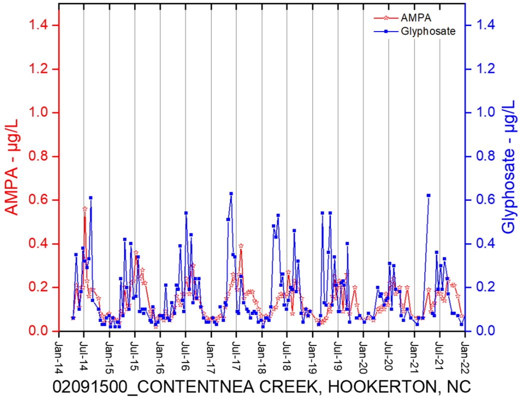
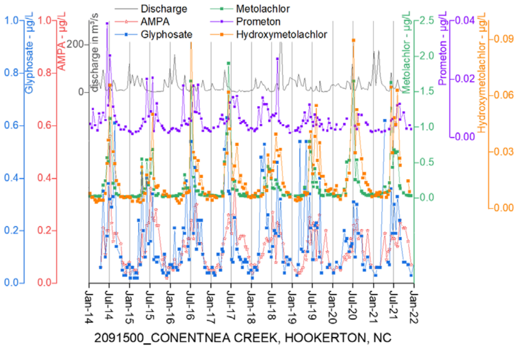
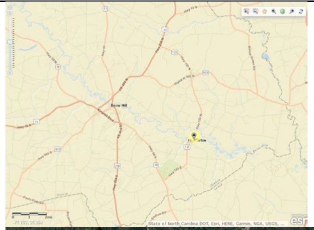
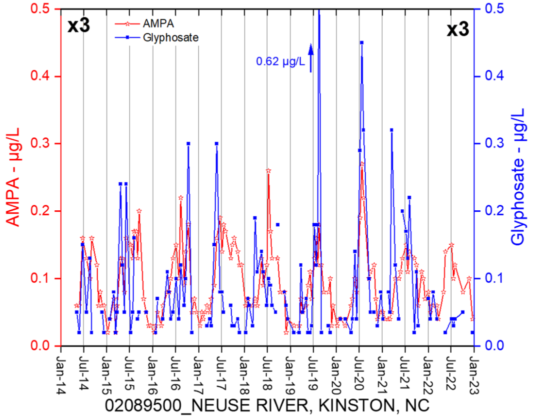
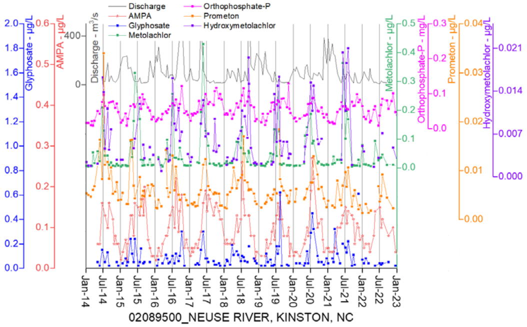
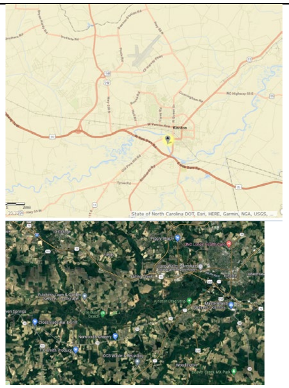
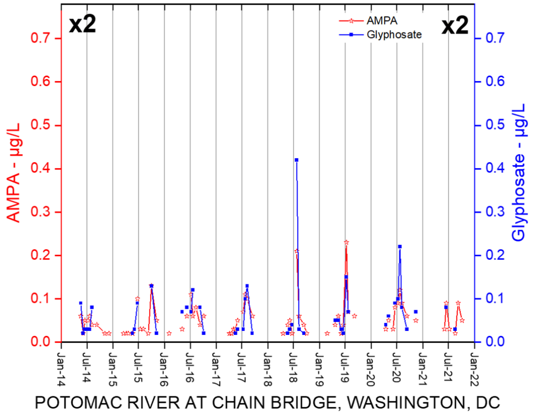
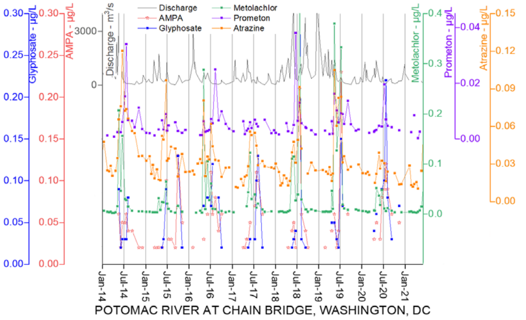
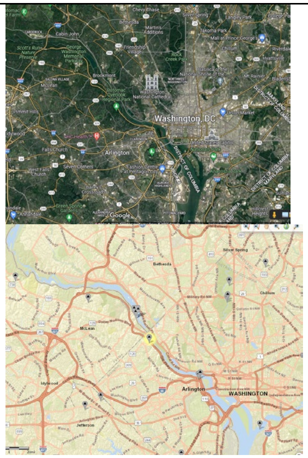
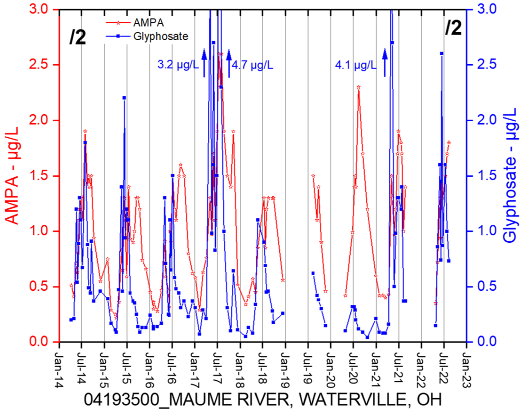
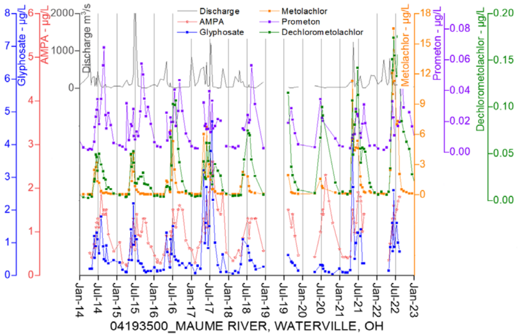
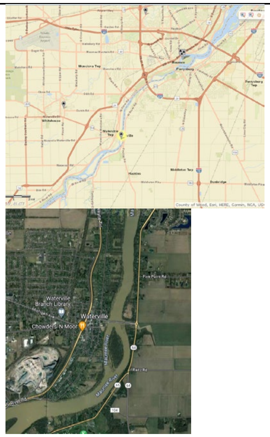
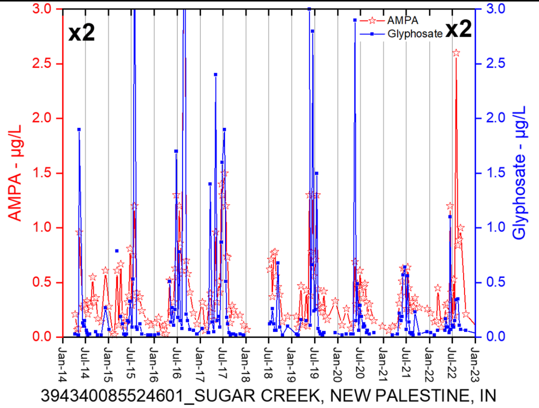
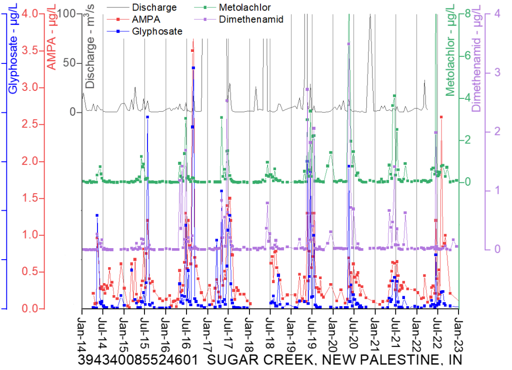
U12 GA	**site:** 02335870_SOPE CREEK NEAR MARIETTA, GA **catchment:** 79 km² fully urban catchment in Marietta, living area with large single houses and a high percentage of sealed surface **WWTP:** no WWTP			 
U13 NC	**site:** 02091500_ CONTENTNEA CREEK AT HOOKERTON, NC **catchment:** 1,879 km² agricultural catchment with forests **WWTP:** Hookerton close to sampling site (400 inh.), Snow Hill (1,500 inh.)			 

U14 **NC**	**site:** 02089500_NEUSE RIVER AT KINSTON, NC **catchment:** 6,899 km² agriculture with city of Kinston **WWTP:** Goldsboro (36,000 inh., UV disinfection)			
U15 **--**	**site:** 01646580_ POTOMAC RIVER AT CHAIN BRIDGE, AT WASHINGTON, DC **catchment:** 29,652.5 km² forests and fields upstream; sampling site urban **WWTP:** one larger WWTP (no information on disinfection)			

U16 OH	**site:** 04193500_ MAUME RIVER AT WATERVILLE OH **catchment:** site 16,223 km² upstream of Toledo; intensive agricultural land use **WWTP:** Napoleon (8,900 inh., UV disinfection), Defiance (17,000 inh., UV disinfection)			
U17 IN	**site:** 39434008552 4601_SUGAR CREEK AT CO RD 400 S AT NEW PALESTINE, IN **catchment:** 237 km² agricultural land use, several smaller villages **WWTP:** no WWTP			

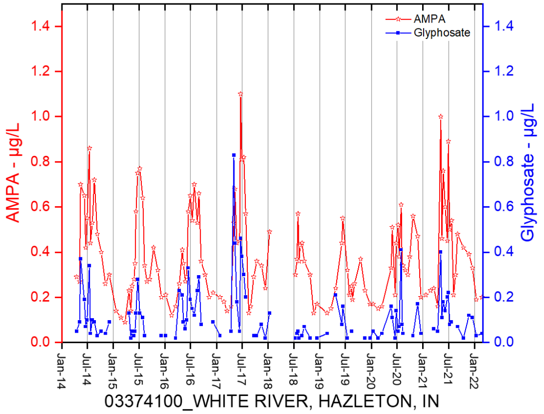
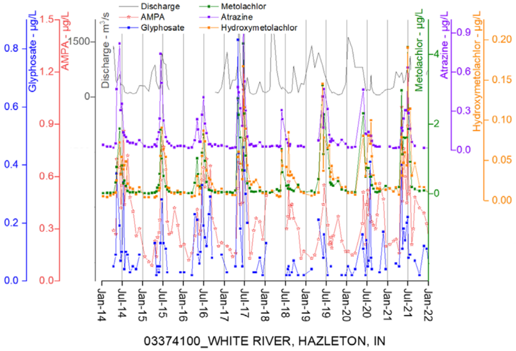
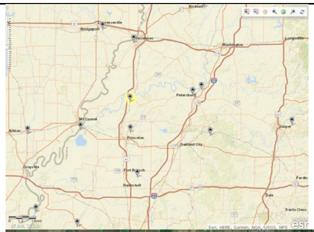
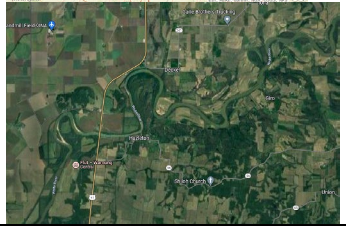
U18 **IN**	**site:** 03374100_WHITE RIVER AT HAZLETON, IN **catchment:** 28,973 km² intense agricultural land use **WWTP:** Petersburg (2,300 inh.)			

*Table S1-2: European sampling sites for France (Départment given in brackets), Sweden, Germany, Italy, The Netherlands, United Kingdom. Site names are taken from the data providers. For Germany, France, The Netherlands and Luxembourg, maps are provided showing the location of the sampling sites. Googlemaps or maps bing aerial view were used, whichever provided better resolution, for some maps, open street map was used. Values <LOD are not included in the plots. In the left column, we use an identical scale for glyphosate and AMPA concentrations, except in rare cases, then the concentration difference where too high (indicated with e.g. *5 for a five time zoom). For WWTPs, the capacity is given as inhabitant equivalents IE when available or number of inhabitants in the city or village.*

Data sources:

***France (FR):** concentration data and map: naiades website during 2023 run by the Office Français de la Biodiversité, www.ofb.gouv.fr, data © at www.ofb.gouv.fr, WWTP information and locations of WWTPs from https://www.assainissement.developpement-durable.gouv.fr/PortailAC/data, discharge data from https://www.hydro.eaufrance.fr/rechercher/entites-hydrometriques*

***Sweden* (SE):** *data obtained from website SLU Jordbruksvatten Version 2022:3, winter data for glyphosate and AMPA for sites M42 and N34 kindly provided by mail from Sveriges lantbruksuniversitet and Swedish University of Agricultural Sciences.*

***Germany* (DE):**

WWTP information: https://kommunales-abwasser.de/ and website of service providers or newspapers for very small WWTPs

- *Baden-Württemberg (BW): data provided by Landesanstalt für Umwelt Baden-Württemberg and website Daten- und Kartendienst der LUBW, https://udo.lubw.baden-wuerttemberg.de, discharge data Mannheim provided by Wasserstraßen- und Schifffahrtsamt Neckar*
- *North Rhine-Westphalia (NRW): data taken from the website https://www.elwasweb.nrw.de/*
- *Rhineland-Palatinate (Rhp): data taken from the website https://geoportal-wasser.rlp-umwelt.de or provided by mail from the Umweltamt Rheinland-Pfalz,*
- *Thuringia (Thu): data kindly provided by TLUBN by mail, data other than for glyphosate were only available for the years 2020-2022,*
- *Bavaria (Bay): data sent by the Landesamt für Umwelt Bayern, map: https://www.nid.bayern.de/wassertemperatur/unterer_main/kahl-a-main-messstation-24078008/stammdaten*
- *Hessen: data kindly provided by Hessisches Landesamt für Naturschutz, Umwelt und Geologie by mail*
- *Berlin (Ber): glyphosate and AMPA data sent by mail by Berliner Wasserbetriebe; other concentration data provided by Senatsverwaltung für Mobilität, Verkehr, Klimaschutz und Umwelt, Berlin by mail;*
  discharge data available online at the Wasserportal Berlin (https://wasserportal.berlin.de/station.php?anzeige=d&station=5870100&thema=odf), Site Lichterfelde

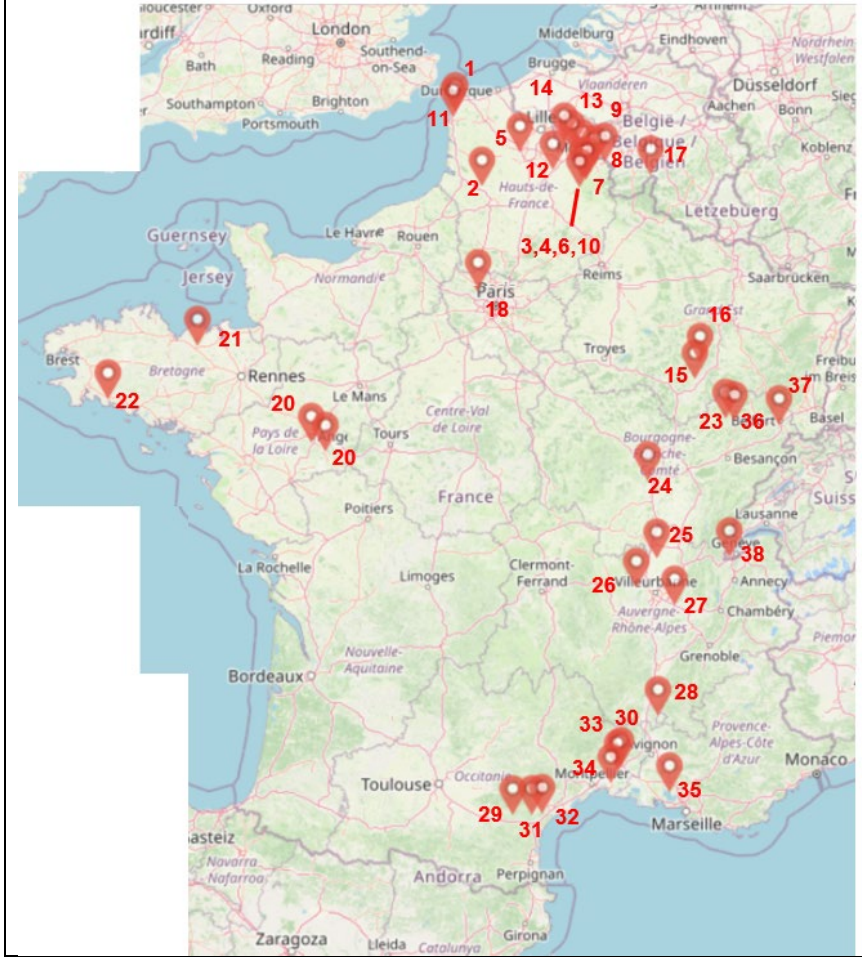
***The Netherlands* (NL):** *data from the website https://www.waterkwaliteitsportaal.nl/oppervlaktewaterkwaliteitin 2023; map data from https://www.msimons.nl/osm/; WWTP information: https://watersector.nl/rwzi/101/rwzi*

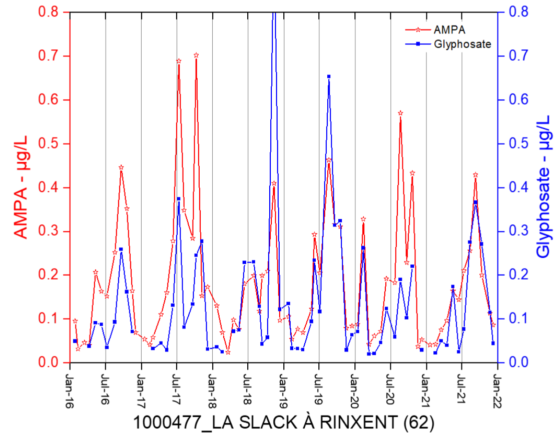
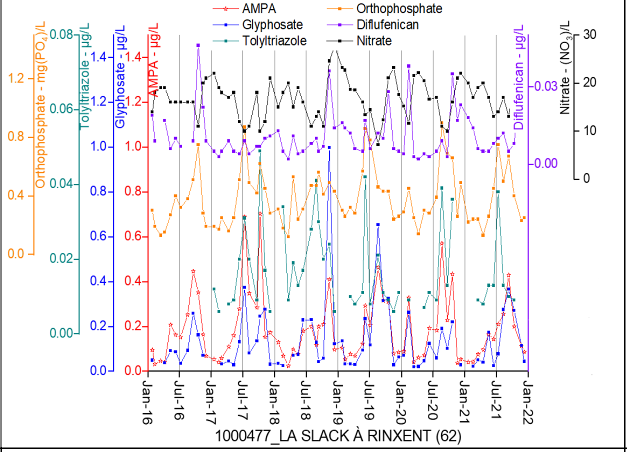
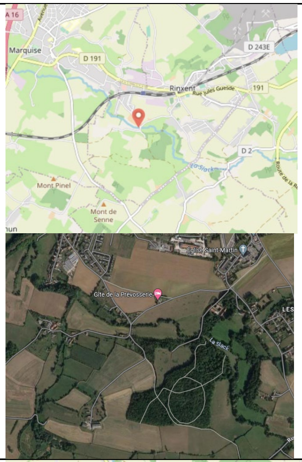
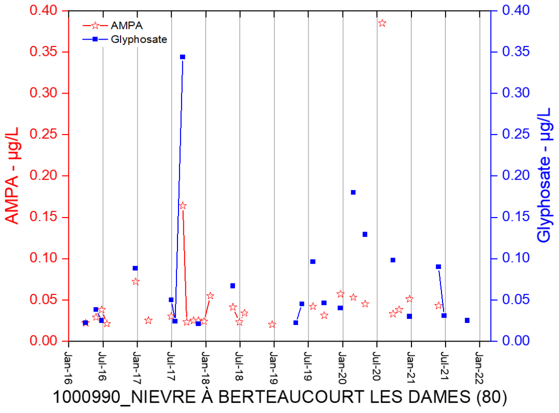
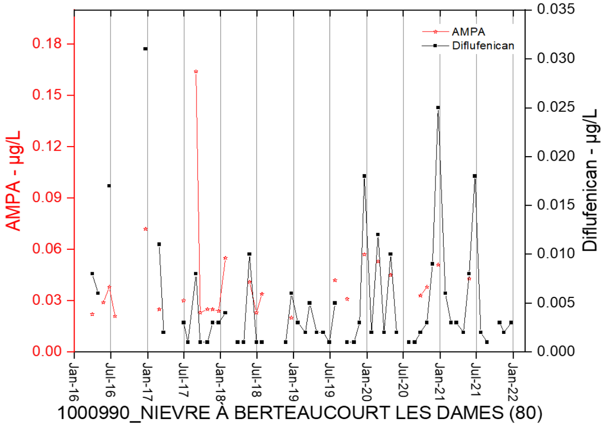
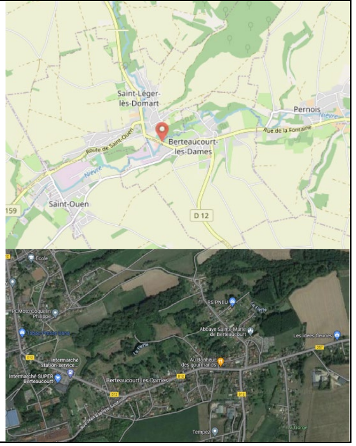
***Italy* (IT):** *data taken from ARPA Toscana, https://www.arpat.toscana.it/documentazione/catalogo-pubblicazioni- arpat/il-controllo-dei-depuratori-di-acque-reflue-urbane-nel-2020; map: https://sira.arpat.toscana.it/sira/opendata/preview.php?dataset=NIT_STATO*

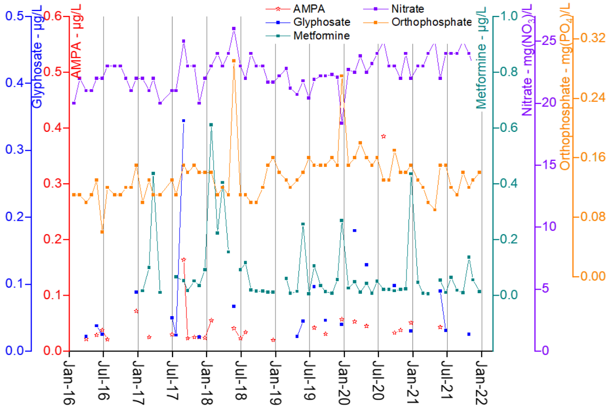
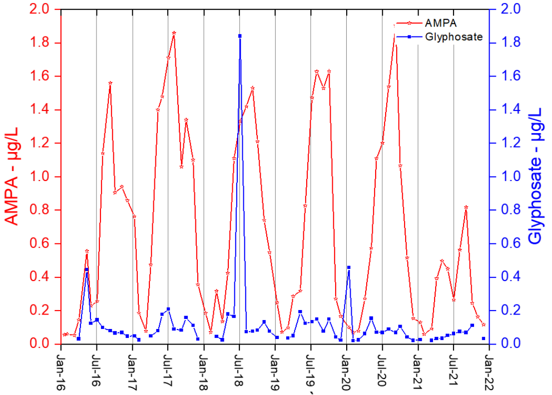
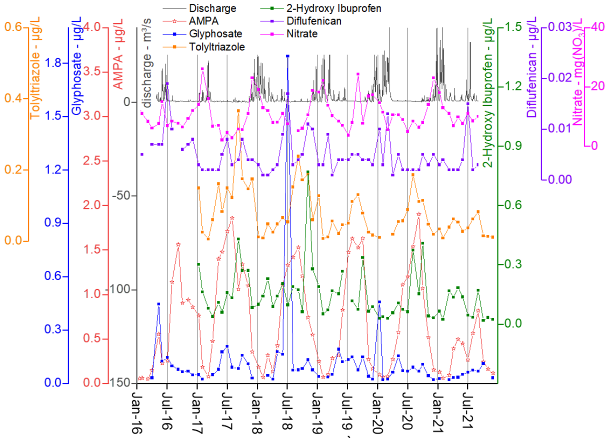
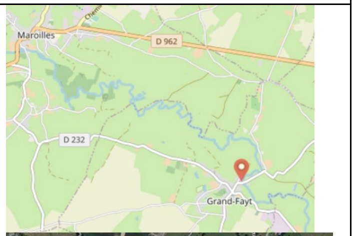
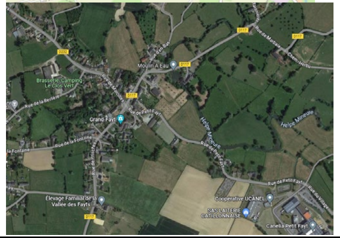
***United Kingdom* (UK):** *data downloaded from the website https://environment.data.gov.uk/water-quality/view/landing in 2023*

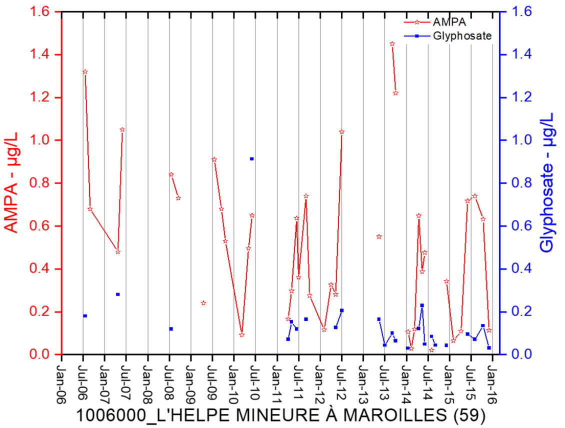
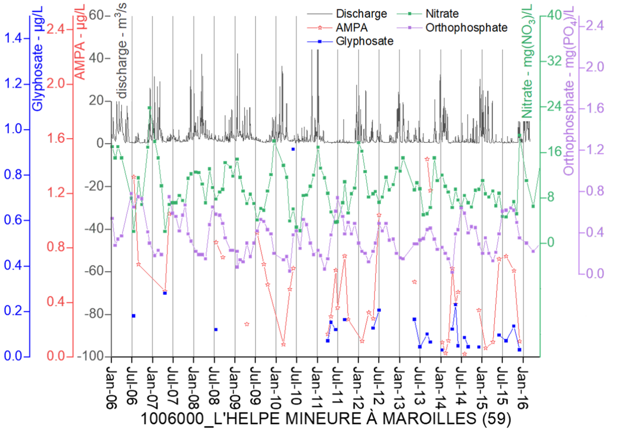
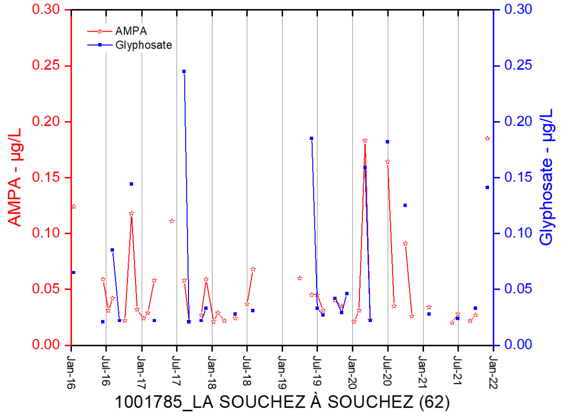
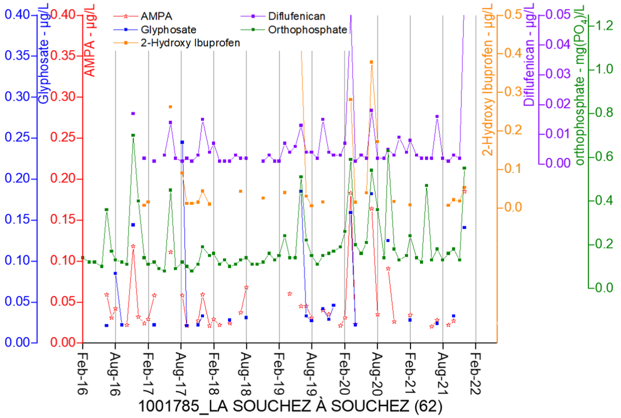
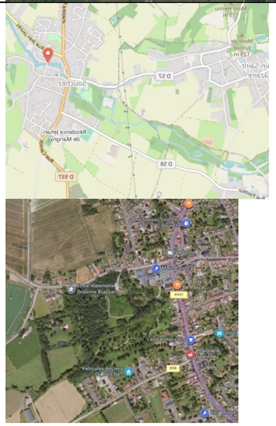
***Luxembourg* (LU):** *all data kindly provided by mail by the Ministère de l'Environnement, du Climat et du Développement durable, Administration de la gestion de l'eau; WWTP data taken from https://map.geoportail*

Site	Site name and description	Seasonal patterns for glyphosate and AMPA	Comparison to agricultural and wastewater-derived micropollutants, orthophosphate, nitrate and/or discharge	site location and aerial view
	France			



E1 **FR** **(62)**	**site:** 1000477_LA SLACK À RINXENT **catchment:** 38 km²; agricultural fields upstream, no larger city, several small separate farms **WWTP:** no WWTP, possibly wastewater impact from private sewers			
E2 **FR** **(80)**	**site:** 1000990_NIEVRE À BERTEAUCOURT LES DAMES **catchment:** 269 km² (Flixecourt); mixed urban-agricultural (fields), several villages **WWTP:** WWTP in a tributary upstream, 320 IE			

			 1000990_NIEVRE À BERTEAUCOURT LES DAMES (80)	
E3 **FR** **(59)**	**site:** 1001131_HELPE MINEURE À GRAND FAYT **catchment:** 263 km² (Maroilles); high agricultural use, grassland, fields; sparsely populated **WWTP:** dairy factory with WWTP, Station de GRAND-FAYT (56 IE), Station de CARTIGNIES (200 IE), Station de ETROEUNGT (650 IE), Station de FOURMIES (8,700 IE) **discharge data:** discharge data at Maroilles	 1001131_HELPE MINEURE À GRAND FAYT (59)	 1001131_HELPE MINEURE À GRAND FAYT (59)	 

E4 **FR** **(59)**	**site:** 1006000_L'HELPE MINEURE À MAROILLES tributary to Sambre **catchment:** 263 km²; agriculture and smaller cities **WWTP:** Station de MAROILLES (750 IE) **discharge data: D013 7020 01** - L'Helpe Mineure à Maroilles (before WWTP discharge point)	 1006000_L'HELPE MINEURE À MAROILLES (59)	 1006000_L'HELPE MINEURE À MAROILLES (59)	
E5 **FR** **(62)**	**site:** 1001785_LA SOUCHEZ À SOUCHEZ **catchment:** small, villages and agriculture (fields) **WWTP:** Station de VILLERS AUX BOIS (EGLISE) SE (233 IE)	 1001785_LA SOUCHEZ À SOUCHEZ (62)	 1001785_LA SOUCHEZ À SOUCHEZ (62)	

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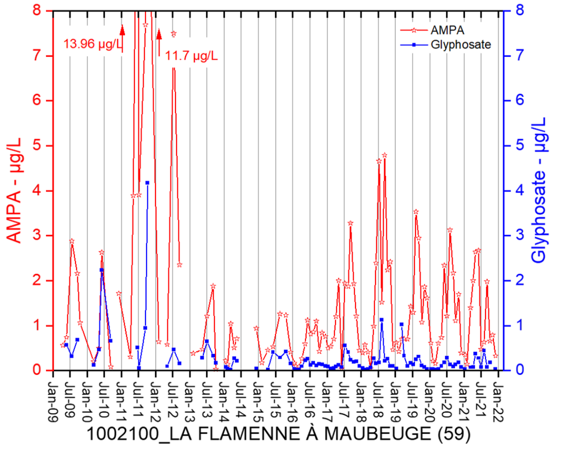
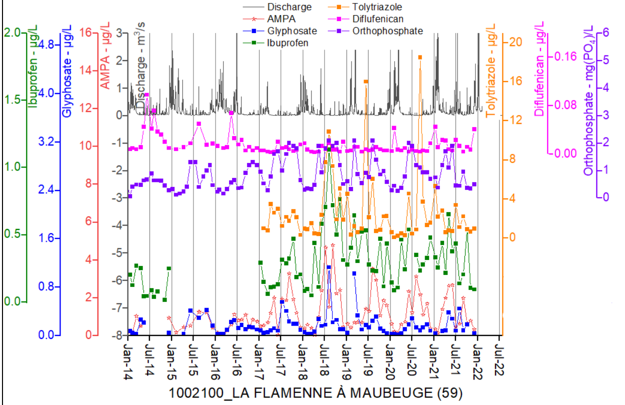
<td>E6
FR
(59)</td>
<td>site: 1002100_LA FLAMENNE À MAUBEUGE tributary to Sambre (site E11)
catchment: 19 km²; small, mainly urban (Maubeuge (26,000 inh.) and Hautmont (14,000 inh.)
WWTP: no WWTP
discharge data: D019 7010 01 - La Flamenne à Maubeuge</td>
<td> </td>
<td> </td>
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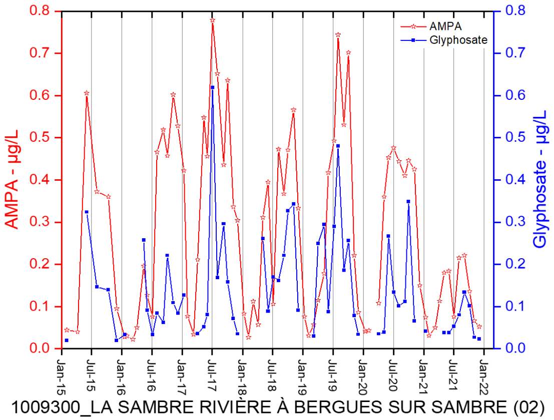
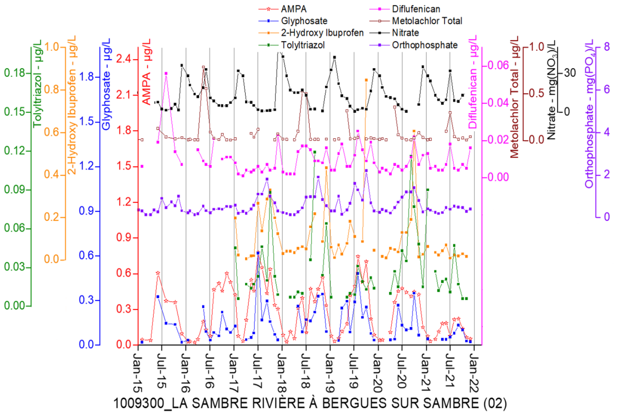
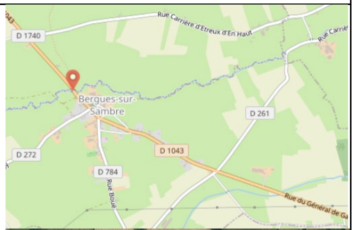
FR
(02)</td>
<td>site: 1009300_LA SAMBRE RIVIÈRE À BERGUES SUR SAMBRE
catchment: ca. 15 km²; agriculture (fields, grassland), small villages
WWTP: presumably small private sewage systems</td>
<td> </td>
<td> </td>
<td> </td>
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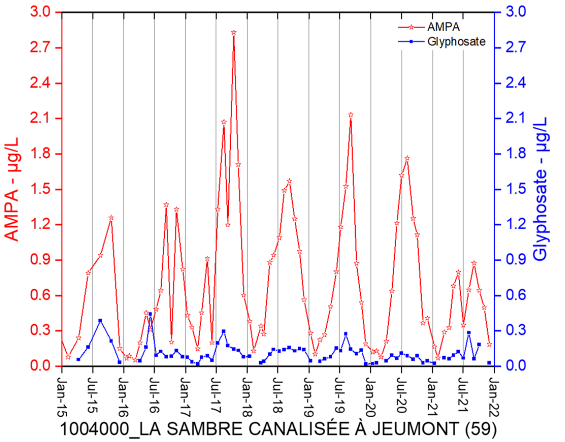
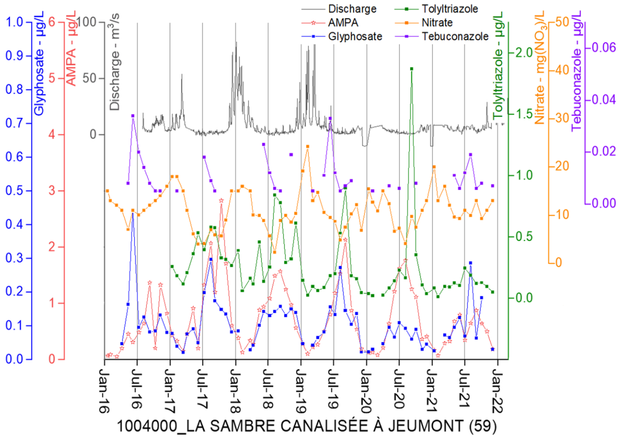
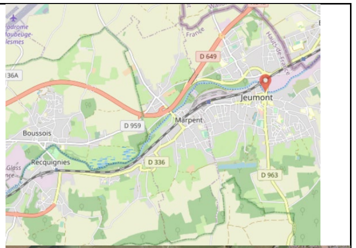
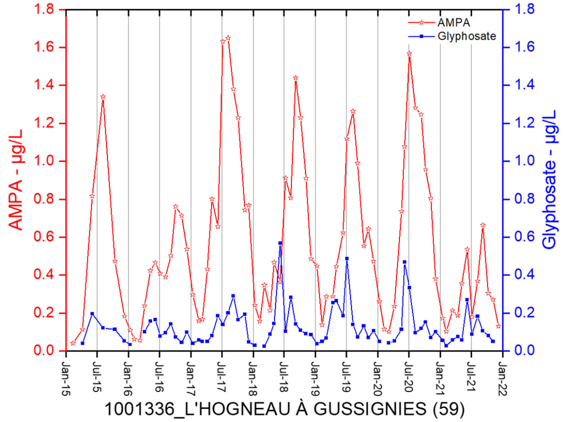
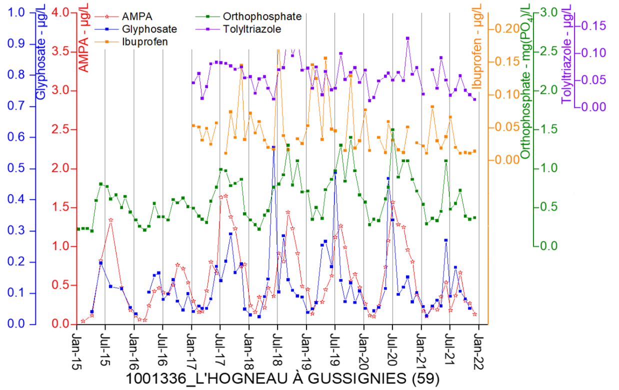
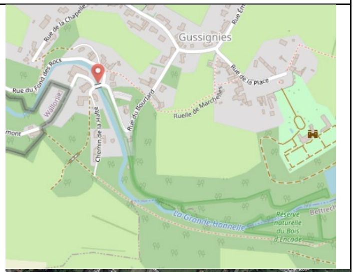
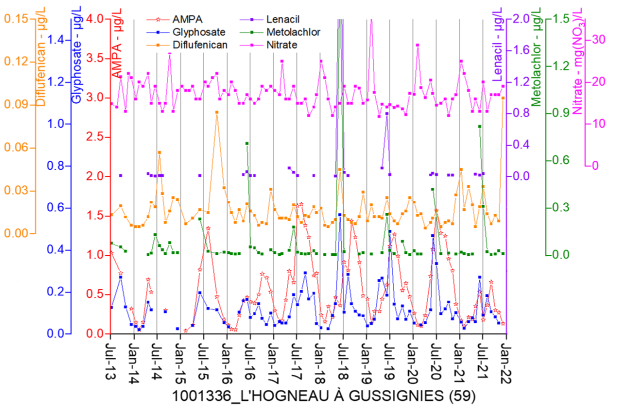
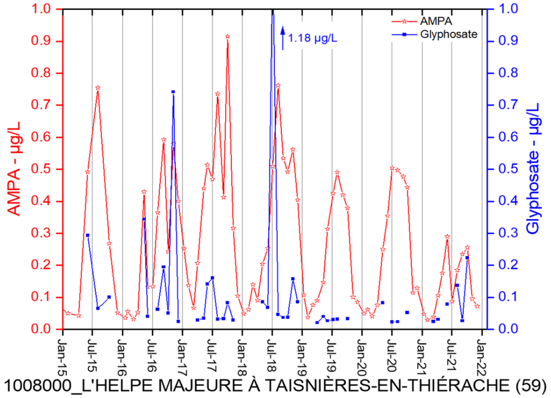
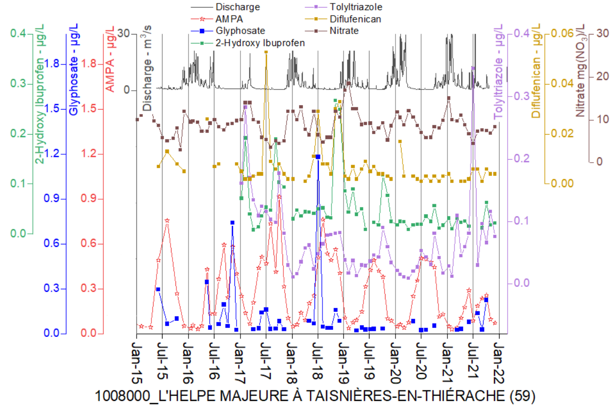
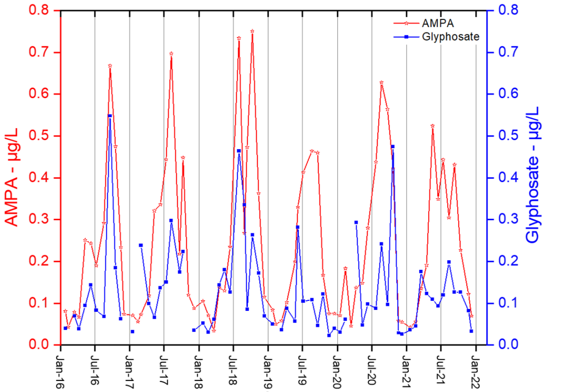
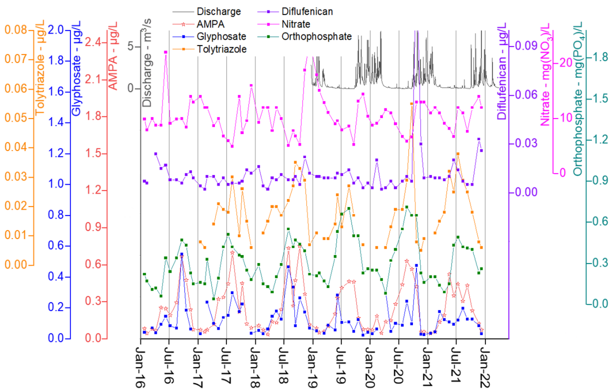
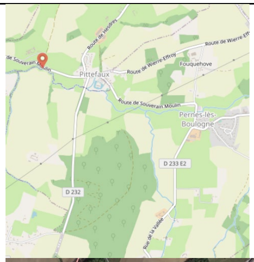
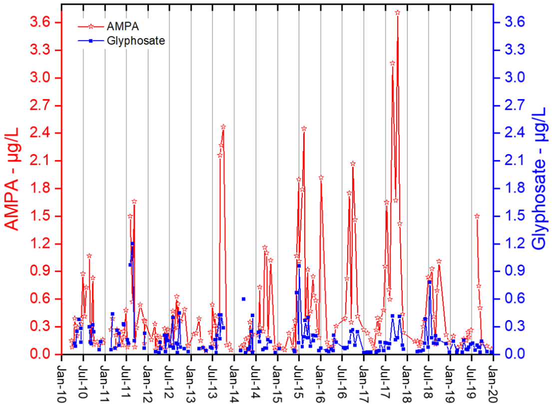
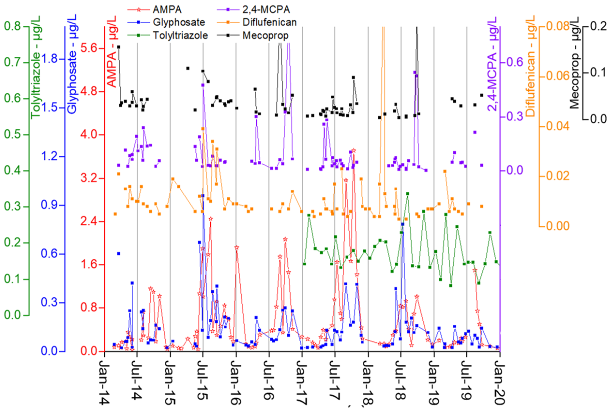
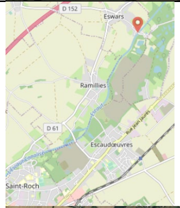
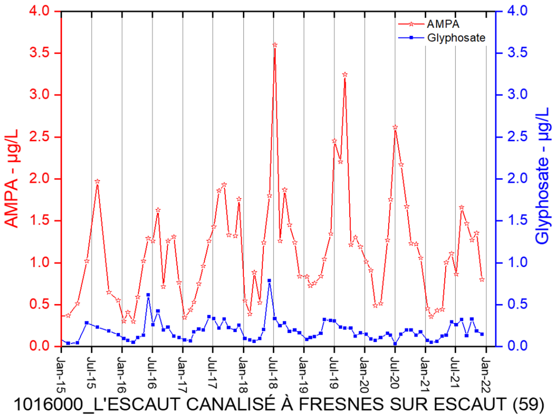
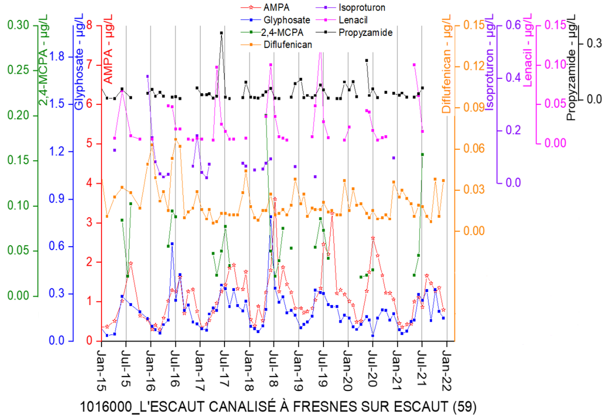
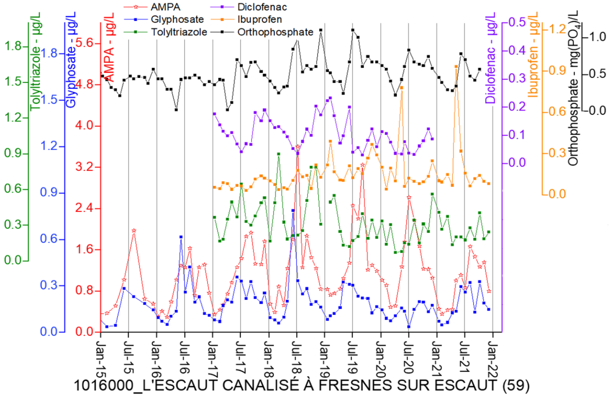
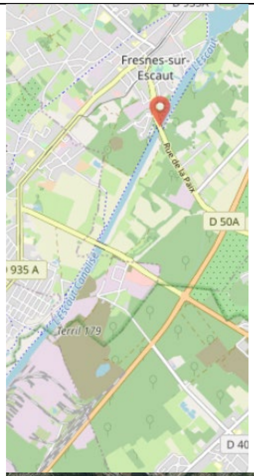
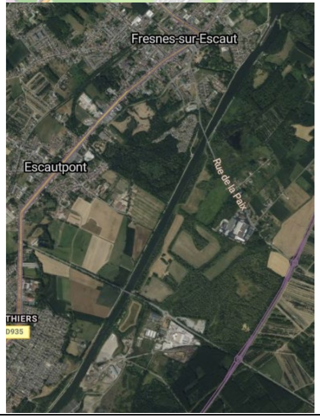
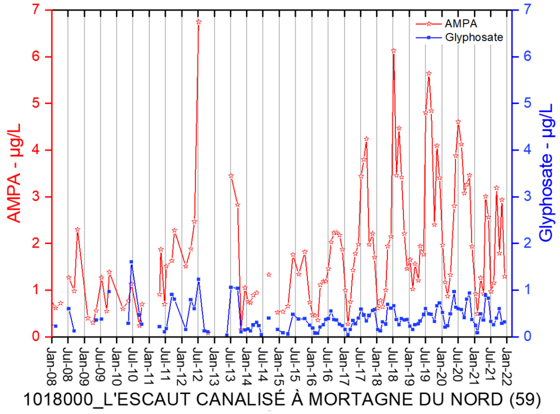
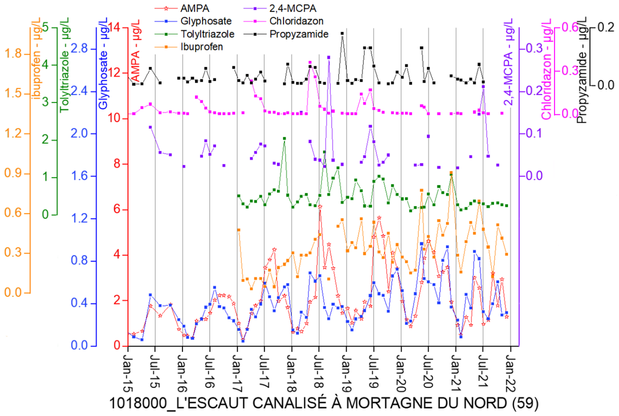
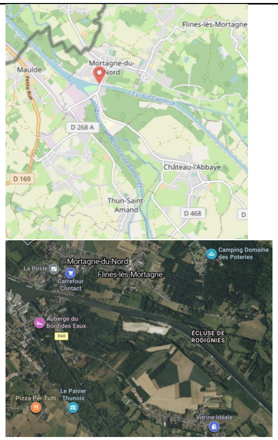
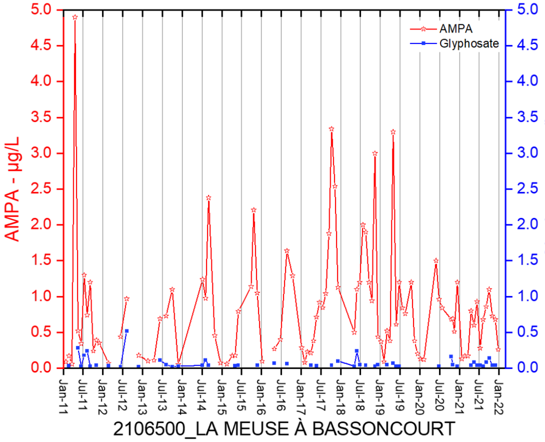
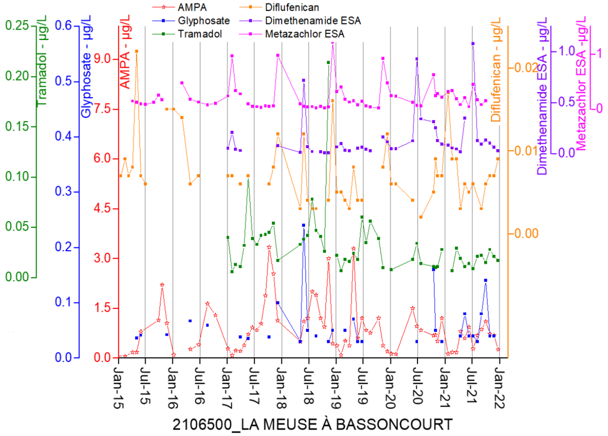
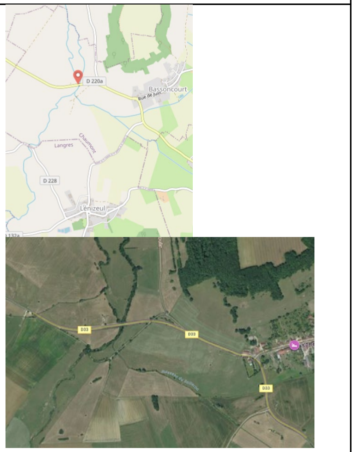
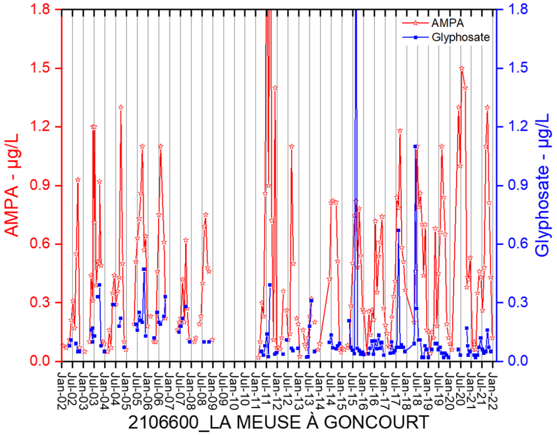
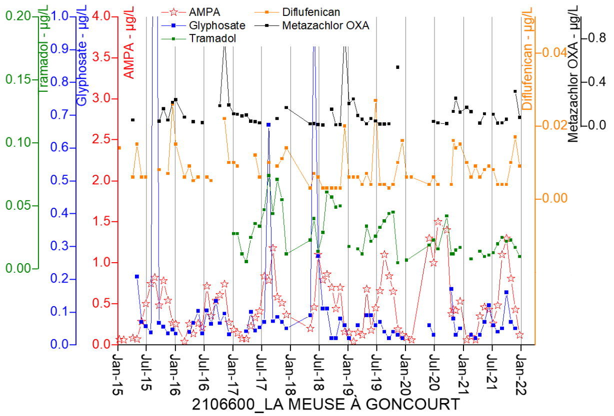
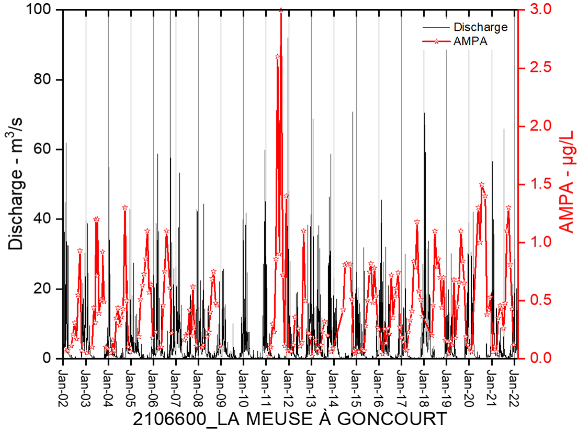
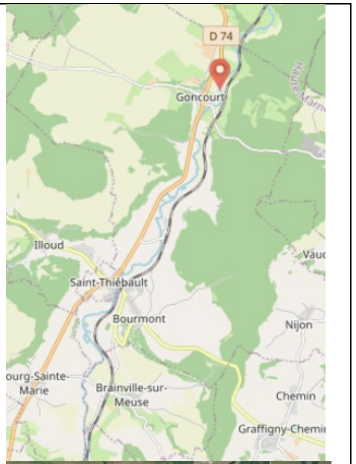
E8 **FR** **(59)**	**site:** 1004000_LA SAMBRE CANALISÉE À JEUMONT **catchment:** 1,170 km² (Marpent); mixed urban and agriculture **WWTP:** close to sampling point: Station de JEUMONT, (18,500 IE), Station de MAUBEUGE (96,000 IE) **discharge data: D019 2230 01** - La Sambre canalisée à Marpent			 
E9 **FR** **(59)**	**site:** 1001336_L'HOGNEAU À GUSSIGNIES **catchment:** 240 km² (Thivencelle); fields, forest, some villages **WWTP:** Station de BELLIGNIES (1,100 IE), Station de HON-HERGIES (540 IE), tributary Station de SAINT-WAAST (320 IE), Station de BAVAY (5,500 IE)			 

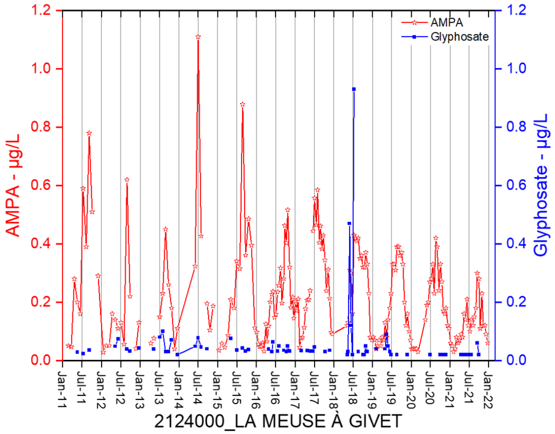
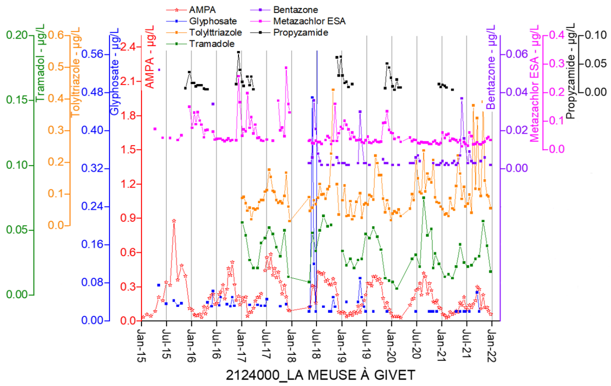
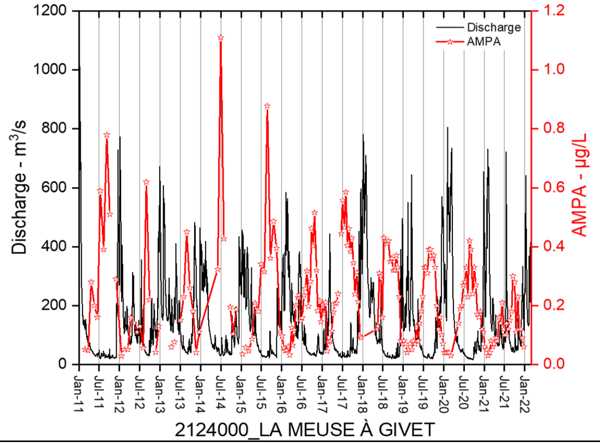
				
E10 **FR** **(59)**	**site:** 1008000_L'HELPE MAJEURE À TAISNIÈRES-EN-THIÉRACHE tributary of Sambre **catchment:** 320 km²; small villages, agriculture (fields, grassland) **WWTP:** Station de DOMPIERRE-SUR-HELPE (275 IE) **discharge data: D015 6520 01** - L'Helpe Majeure à Taisnières-en-Thiérache			 

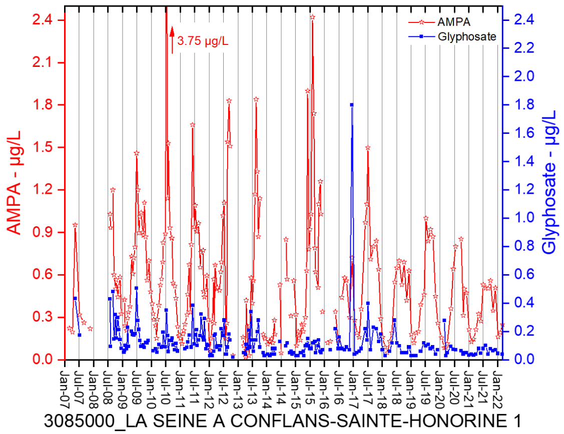
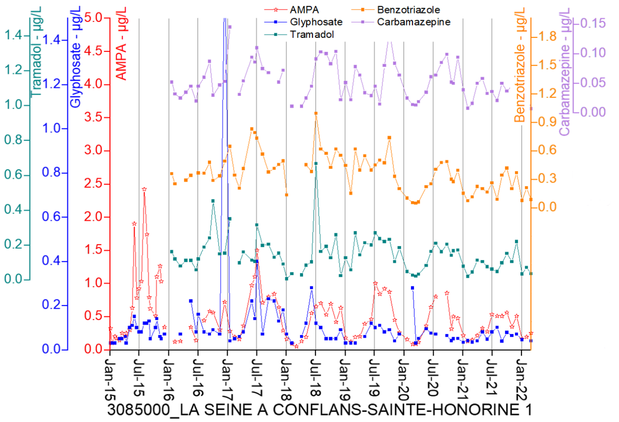
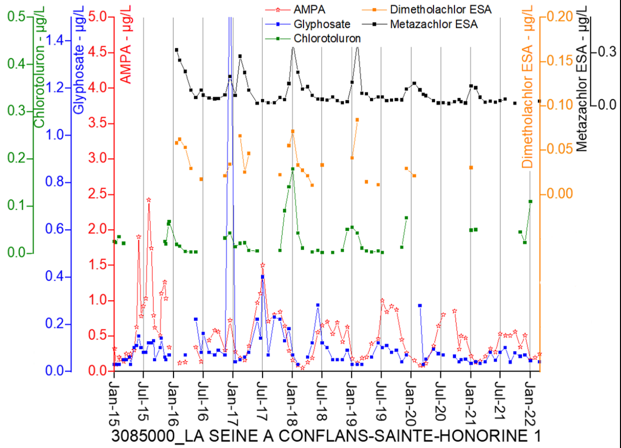
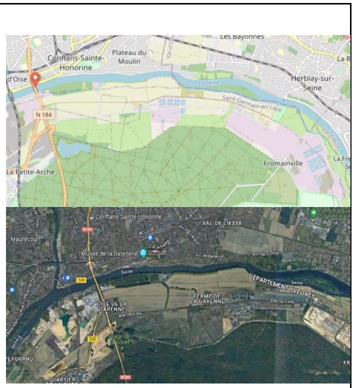
E11 **FR** **(62)**	**site:** 1002229_LE WIMEREUX À MANINGHEN-HENNE **catchment:** high agricultural use, only small villages **WWTP:** Station de PERNES-LES-BOULOGNE (175 IE), Station de CONTEVILLE-LES-BOULOGNE (165 IE) **discharge data:** E520 5712 02 - Le Wimereux à Pittefaux	 1002229_LE WIMEREUX À MANINGHEN-HENNE (62)	 1002229_LE WIMEREUX À MANINGHEN-HENNE (62)	 
E12 **FR** **(59)**	**site:** 1012000_L'ESCAUT CANALISÉ À ESWARS **catchment:** 539 km² (Iwuy); urban and agriculture (fields) several water gates, large sugar factory near sampling site **WWTP:** Station de CAMBRAI (100,000 IE)	 1012000_L'ESCAUT CANALISÉ À ESWARS (59)	 1012000_L'ESCAUT CANALISÉ À ESWARS (59)	 

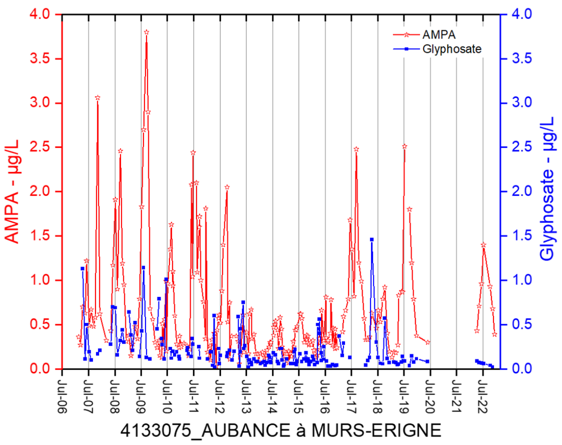
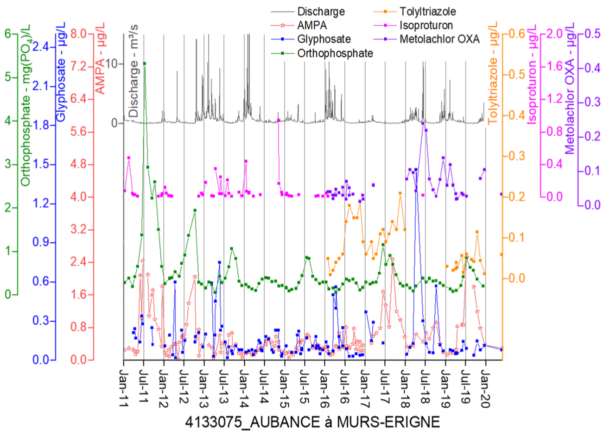
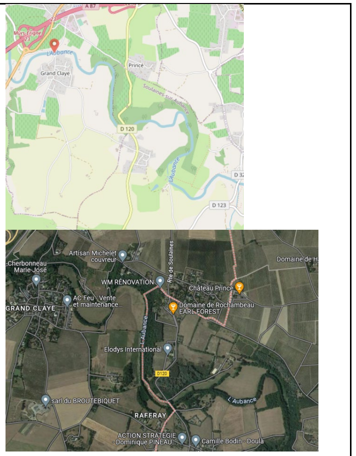
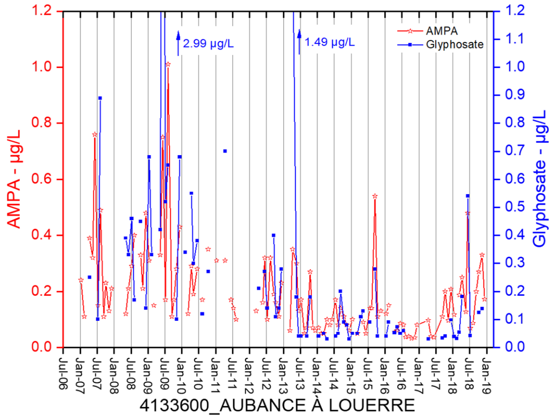
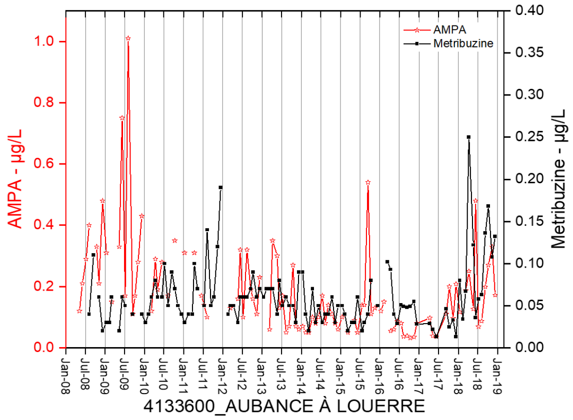
E13 **FR** **(59)**	**site:** 1016000_L'ESCAUT CANALISÉ À FRESNES SUR ESCAUT downstream of site E12 **catchment:** strong urban input, agriculture further upstream **WWTP:** Station de BRUAY-SUR-L'ESCAUT (14,000 IE), Station de RAISMES-BEUVRAGES (40,000 IE)		 	 

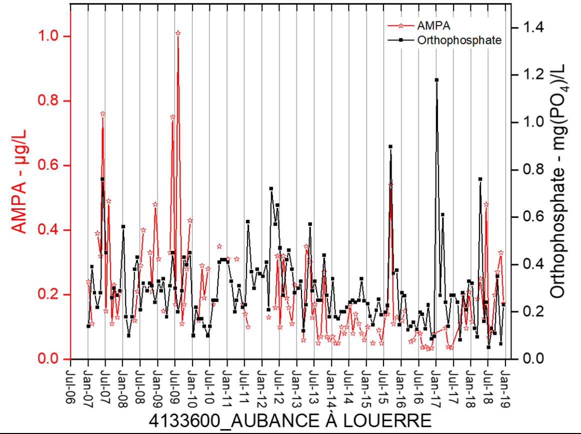
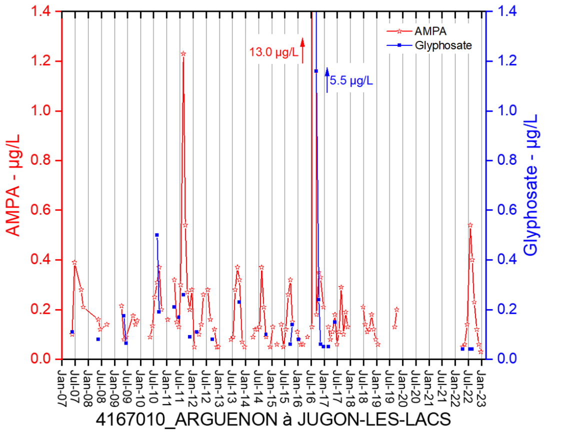
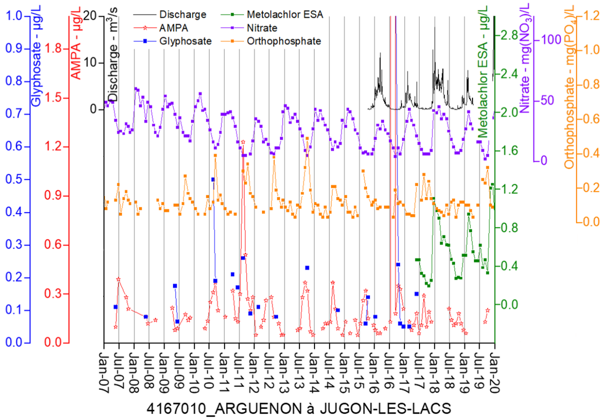
E14 **FR** **(59)**	**site:** 1018000_L'ESCAUT CANALISÉ À MORTAGNE DU NORD; downstream of site E13 **catchment:** 3,503 km²; mixed urban-agricultural **WWTP:** Station de HERGNIES-BRUILLE-SAINT-AMAND (3,300 IE), Station de FRESNES-SUR-ESCAUT (36,000 IE), Station de SAINT-AMAND-LES-EAUX-LECELLES (18,000 IE, tributary)	 1018000_L'ESCAUT CANALISÉ À MORTAGNE DU NORD (59)	 1018000_L'ESCAUT CANALISÉ À MORTAGNE DU NORD (59)	
E15 **FR** **(52)**	**site:** 2106500_LA MEUSE À BASSONCOURT **catchment:** ca. 150 km²; agricultural (grassland, fields), only very small villages **WWTP:** Station de LENIZEUL (20 IE), further small sedimentation ponds and private sewers	 2106500_LA MEUSE À BASSONCOURT	 2106500_LA MEUSE À BASSONCOURT	

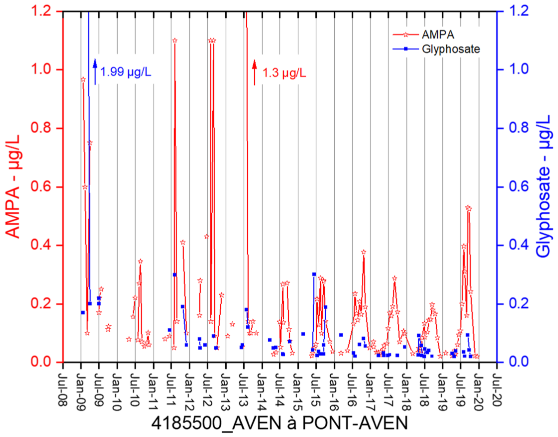
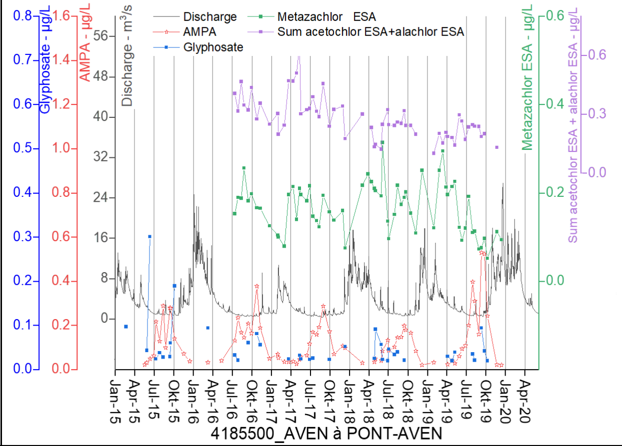
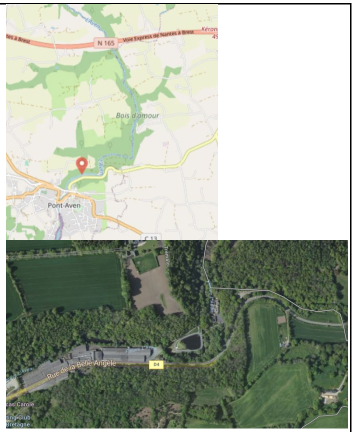
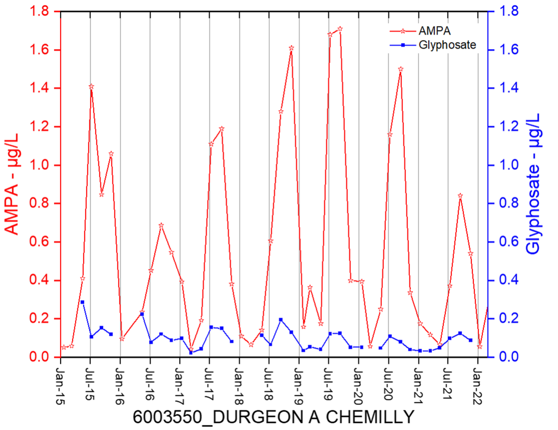
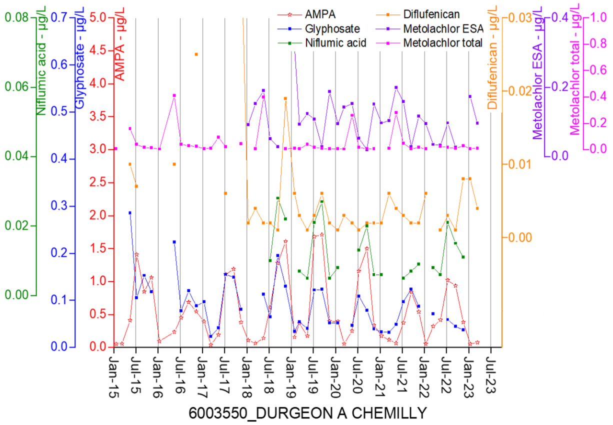
E16 **FR** **(52)**	**site:** 2106600_LA MEUSE À GONCOURT **catchment:** 364 km²; agricultural (grassland, fields), forest, only very small villages **WWTP:** WWTP of a cheese factory in tributary, Station de BOURMONT-ENTRE-MEUSE-ET-MOUZON (41 IE), Station de BOURG-SAINTE-MARIE (90 IE) **discharge data:** B022 0010 01 - La Meuse à Goncourt		 	 

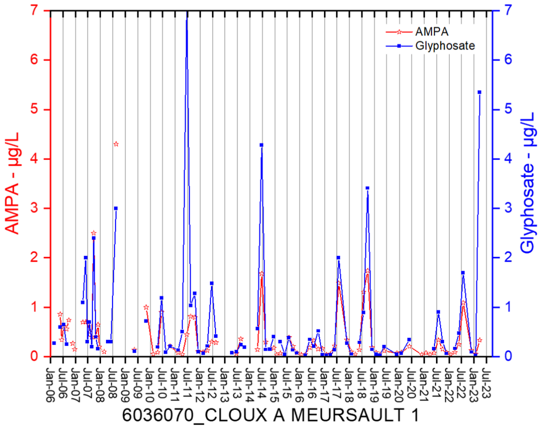
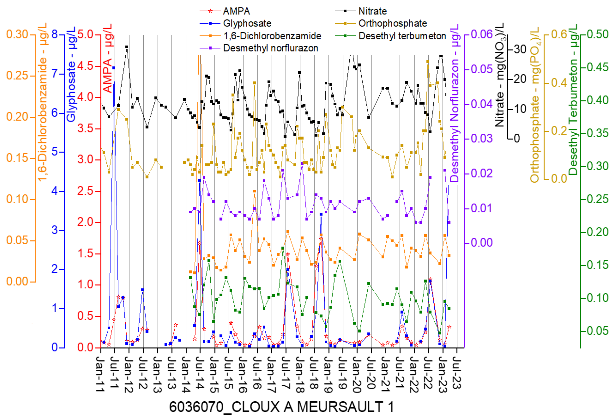
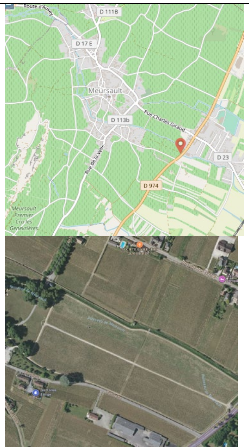
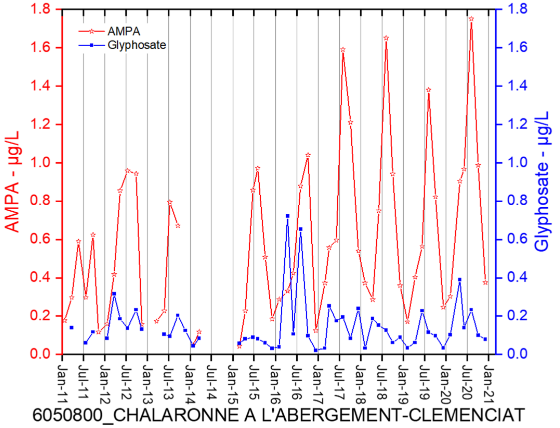
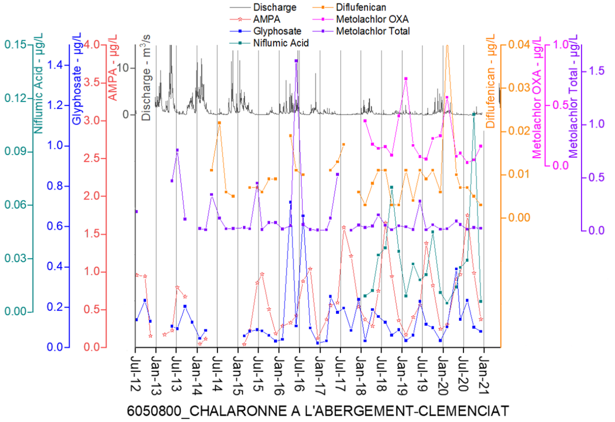
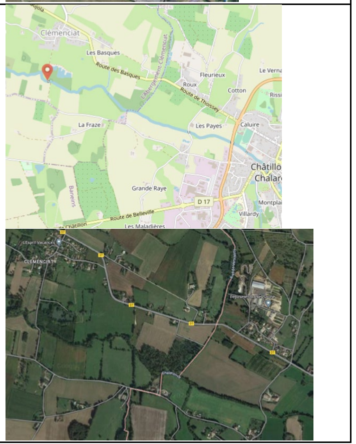
E17 **FR** **(08)**	**site:** 2124000_LA MEUSE À GIVET **catchment:** 10,141 km² (Chooz); mixed urban and agriculture **WWTP:** Station de GIVET (12,000 IE) very close to sampling site **discharge data: B720 0000 01** - La Meuse à Chooz - Trou du Diable		 	 

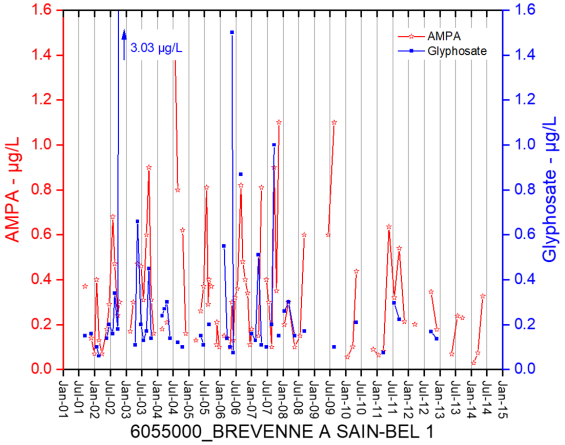
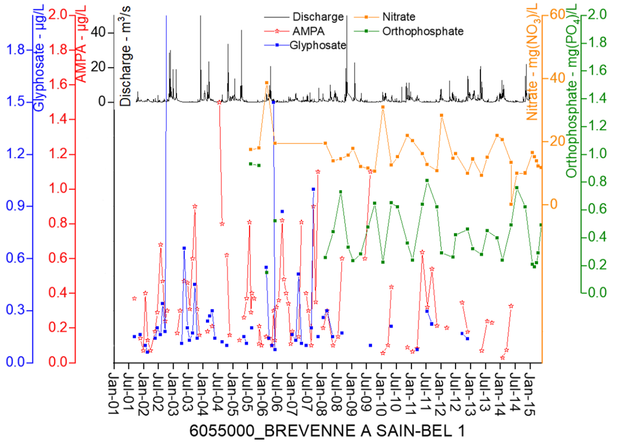
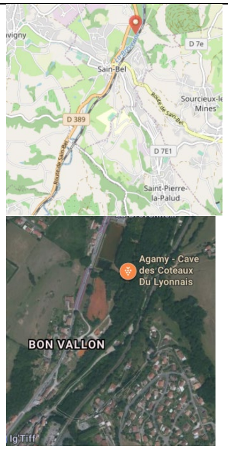
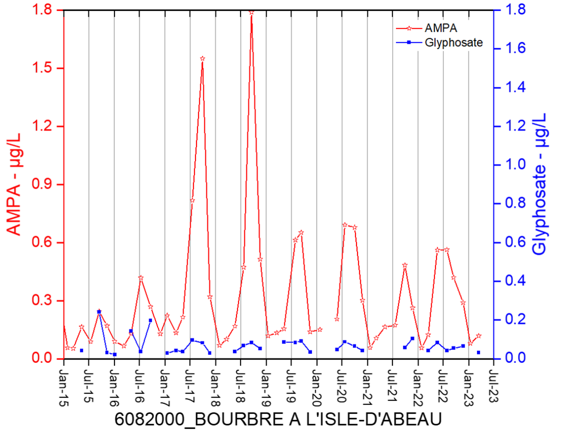
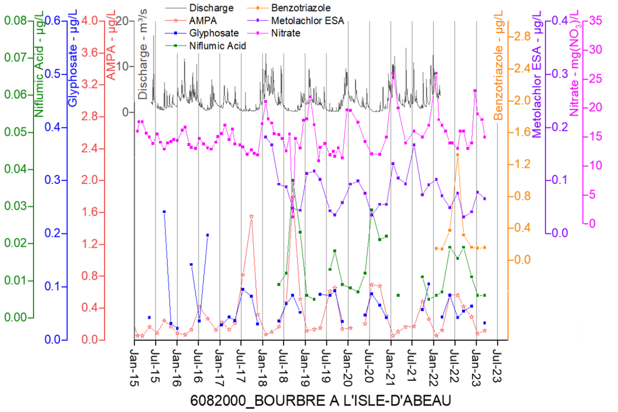
E18 **FR** **(78)**	**site:** 3085000_LA SEINE A CONFLANS-SAINTE-HONORINE 1 **catchment:** 44,669 km²; large urban agglomeration within catchment (Paris); agriculture, winegrowing (Champagne), **WWTP:** Station de PARIS SEINE AVAL (5.4 Mio IE short before sampling point (see map)), WWTP d'Achères without further information		 	

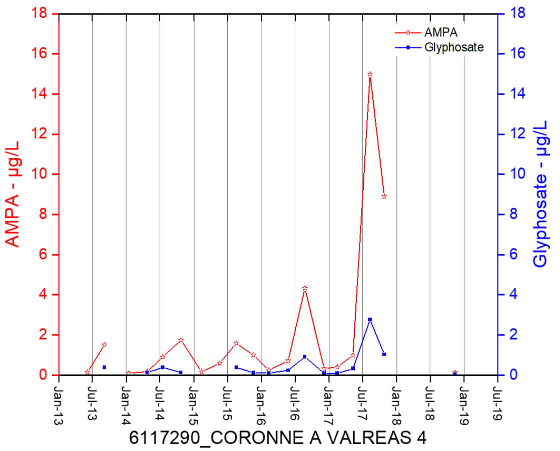
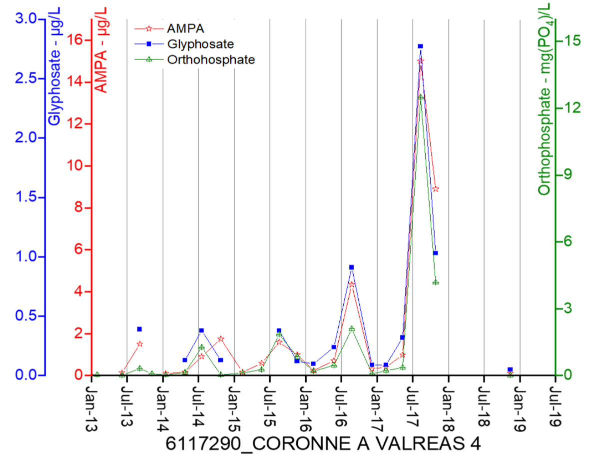
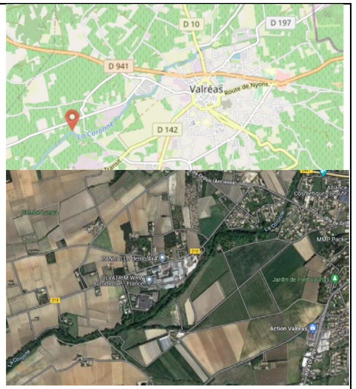
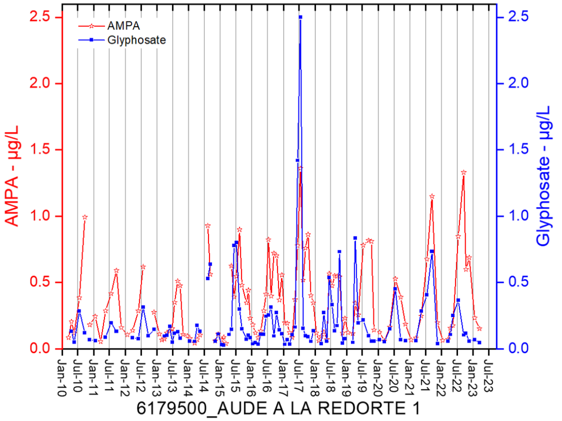
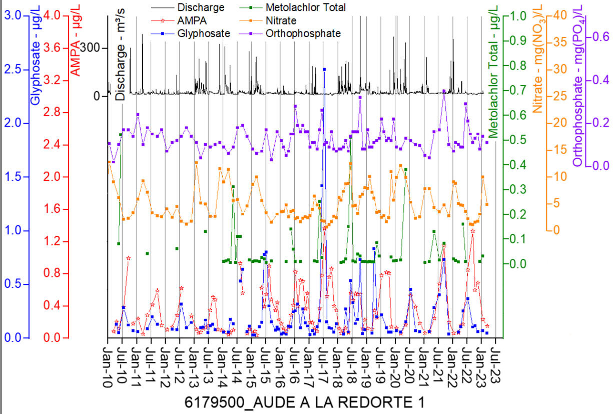
E19 **FR** **(49)**	**site:** 4133075_AUBANCE à MURS-ERIGNE **catchment:** 172 km² (Soulaines-sur-Aubance); agriculture (vineyards, grassland, fields), villages **WWTP:** Station de SOULAINES BALUERE (61 IE), Station de ST MELAINE - LE PONT AUX MOINES (1,400 IE), Station de BRISSAC (3,100 IE) **discharge data:** M501 4210 20 - L'Aubance à Soulaines-sur-Aubance - Charuau			
E20 **FR** **(49)**	**site:** 4133600_AUBANCE À LOUERRE **catchment:** few km²; source of the Aubance in the center of the village Louerre (400 inh.), wastewater treatment ca. 300 m after the source, sampling site ca, 650 m after the source. 3 larger fields between streets and river: 0.08 km² **WWTP:** Station de LOUERRE (160 IE)			

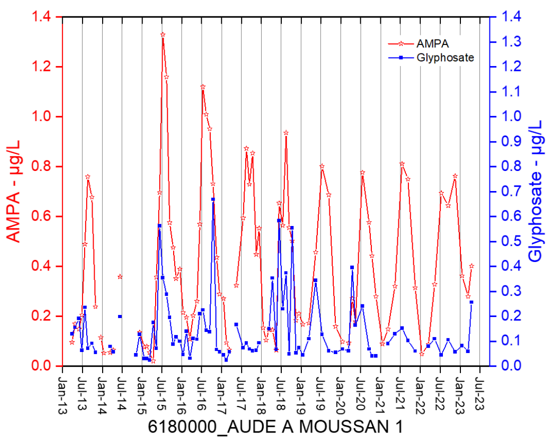
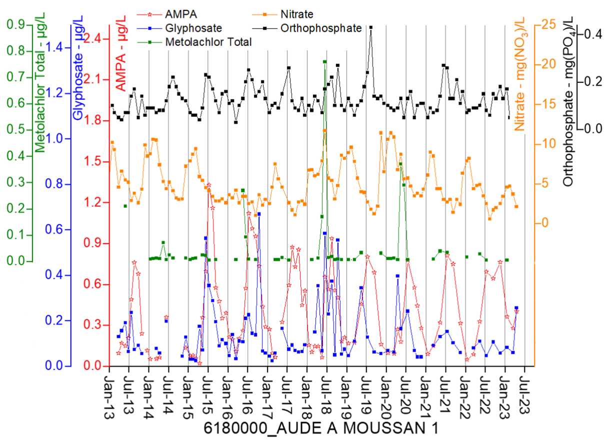
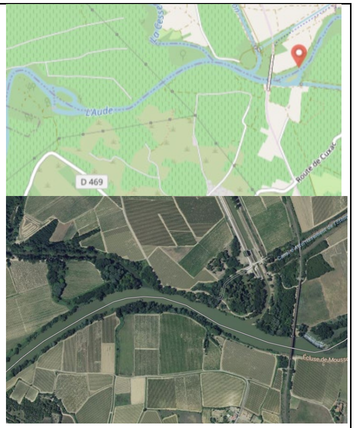
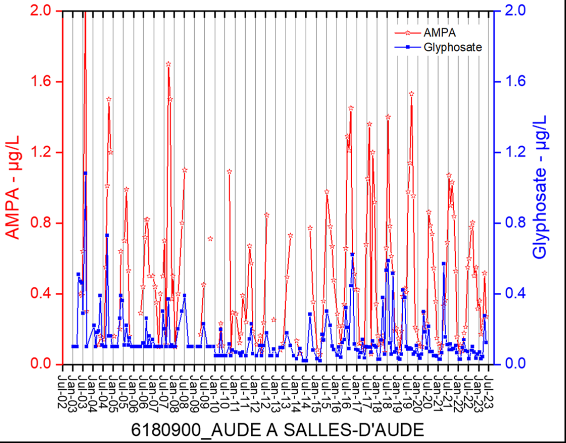
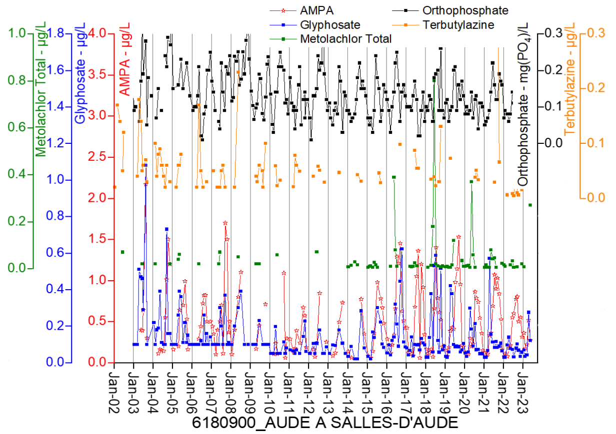
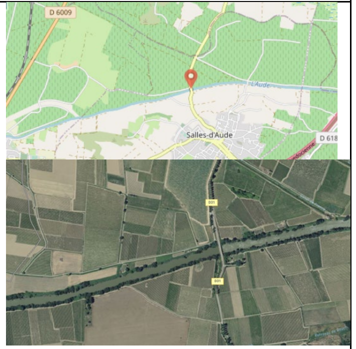
			 4133600_AUBANCE À LOUERRE	
E21 **FR** **(22)**	**site:** 4167010_ARGUENON à JUGON-LES-LACS **catchment:** 318 km²; smaller villages, large fields **WWTP:** Station de JUGON-LES-LACS LE BOUT DE LA VILLE (5,100 IE) very close to sampling site, Station de PLENEE-JUGON BOURG (930 IE) **discharge data:** J112 3020 01 - L'Arguenon à Jugon-les-Lacs - Amont retenue Ville-Hatte	 4167010_ARGUENON à JUGON-LES-LACS	 4167010_ARGUENON à JUGON-LES-LACS	

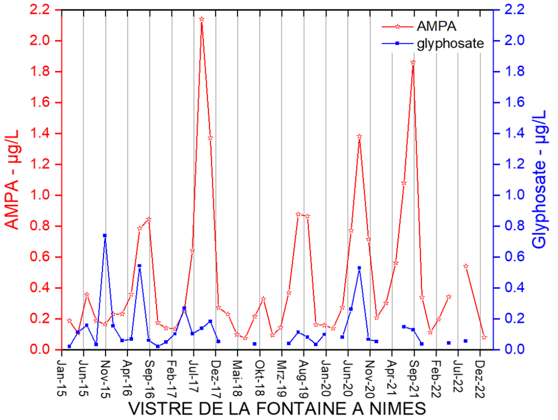
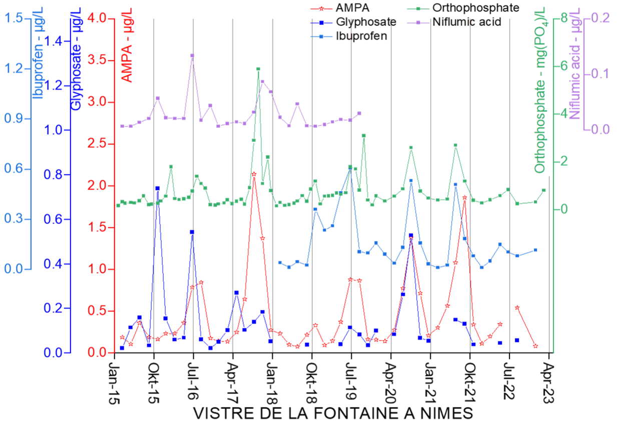
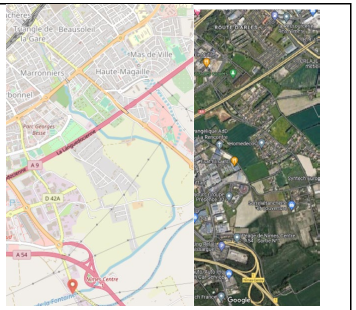
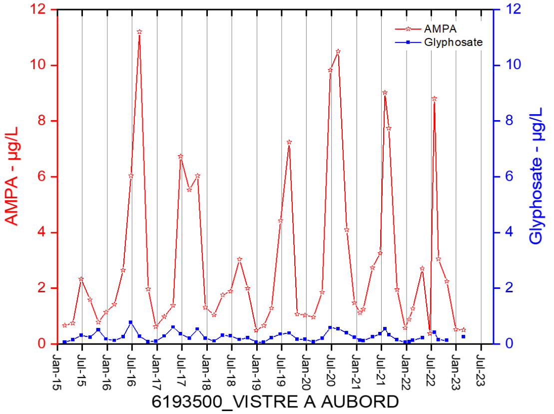
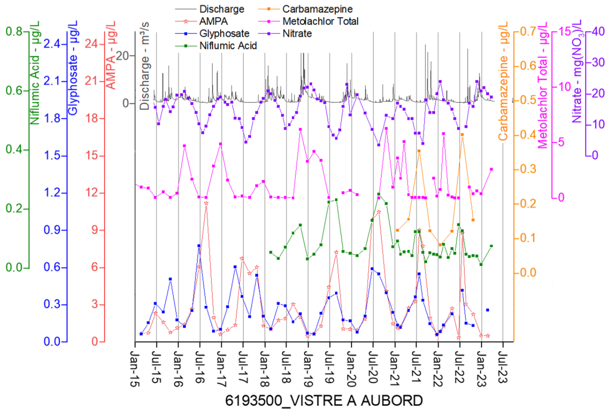
E22 **FR** **(29)**	**site:** 4185500_AVEN à PONT-AVEN **catchment:** 16 km² (Pont-Aven); agricultural (forest, fields) with two cities (Bannalec, Rosporden) **WWTP:** Station de STEU DE: ROSPORDEN – BODUON (27,500 IE), Station de STEU DE: BANNALEC - MOULIN QUINQUIS (31,000 IE, tributary) **discharge data:** L'Aven à Pont-Aven	 4185500_AVEN à PONT-AVEN	 4185500_AVEN à PONT-AVEN	
E23 **FR** **(79)**	**site:** 6003550_DURGEON A CHEMILLY **catchment:** 410 km²; (Pontcey); grassland and fields, only small villages downstream of Vesoul (15,000 inh.) **WWTP:** Station de PONTCEY (1,200 IE), Station de VESOUL NOUVELLE (45,000 IE)	 6003550_DURGEON A CHEMILLY	 6003550_DURGEON A CHEMILLY	

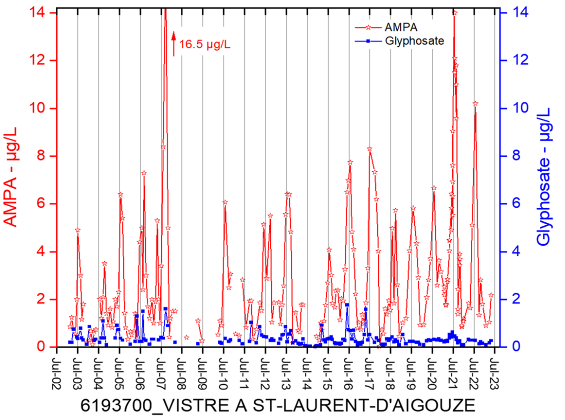
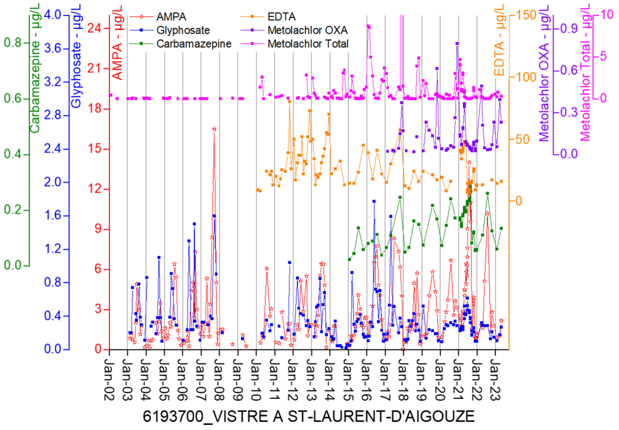
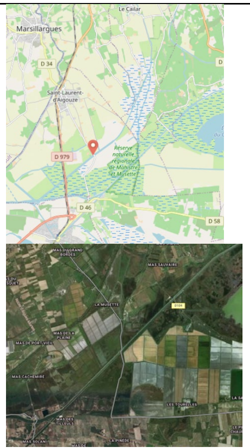
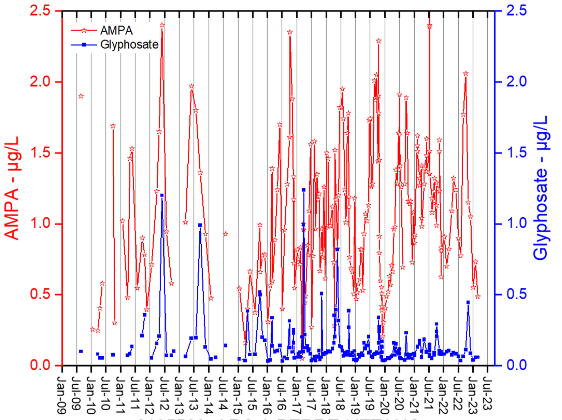
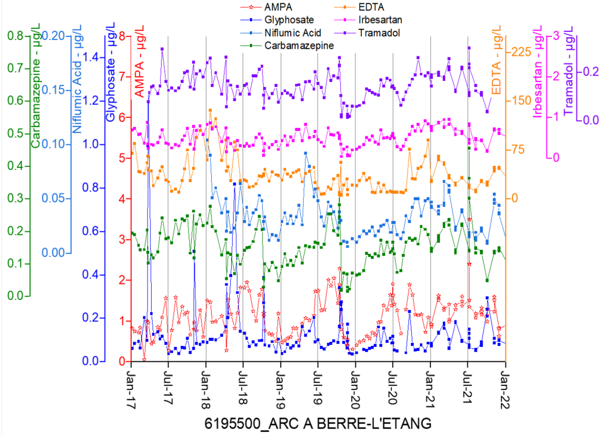
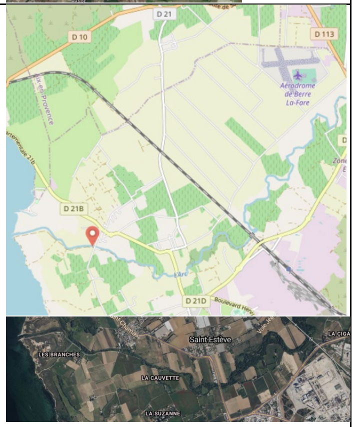
E24 **FR** **(21)**	**site:** 6036070_CLOUX A MEURSAULT 1 **catchment:** vineyards a few villages upstream **WWTP:** Station de SAINT-ROMAIN 21 (75 IE)			
E25 **FR** **(01)**	**site:** 6050800_CHALARONNE A L'ABERGEMENT-CLEMENCIAT **catchment:** 175 km² (Châtillon-sur-Chalaronne); agricultural fields and small city, villages **WWTP:** Station de CHATILLON-SUR-CHALARONNE - CHEF-LIEU (4,500 IE) close to sampling site **discharge data:** Châtillon-sur-Chalaronne			

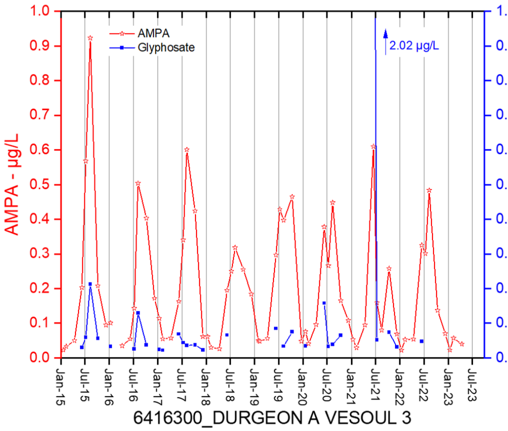
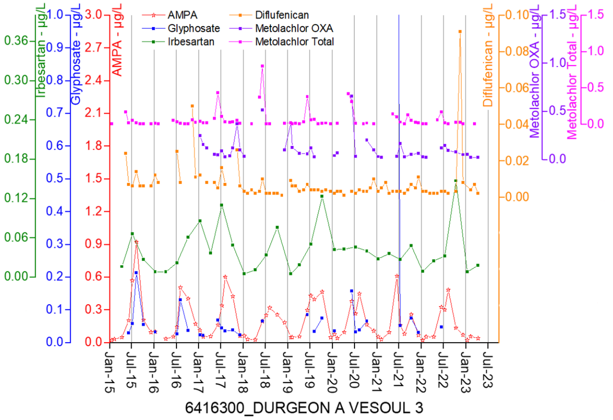
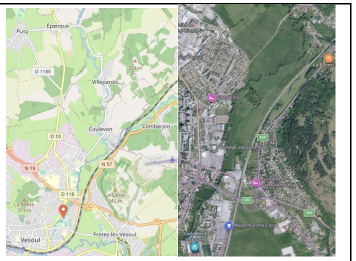
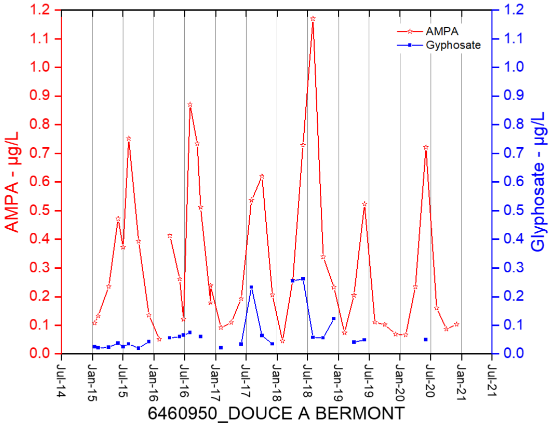
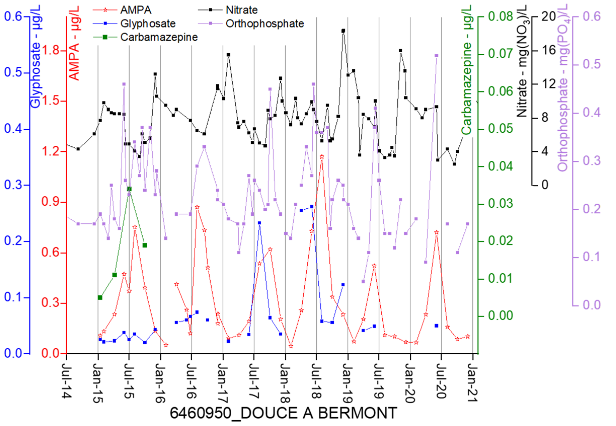
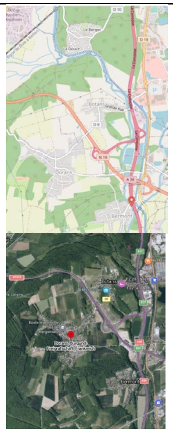
E26 **FR** **(69)**	**site:** 6055000_BREVENNE A SAIN-BEL 1 **catchment:** 219 km²; mixed urban and agriculture (grassland, a few vineyards, fields, train tracks near river **WWTP:** Station de SOURCIEUX-LES-MINES (1,900 IE, tributary), further smaller WWTP <2,200 IE **discharge data:** U463 5010 01 - La Brévenne à Sain-Bel			
E27 **FR** **(38)**	**site:** 6082000_BOURBRE A L'ISLE-D'ABEAU **catchment:** 304 km² (Bourgoin-Jallieu); mixed urban and agriculture (fields, grassland) **WWTP:** Station de BOURGOIN-JALLIEU 2 (84,800 IE) close to sampling site **discharge data:** V173 4010 01 - La Bourbre à Bourgoin-Jallieu			

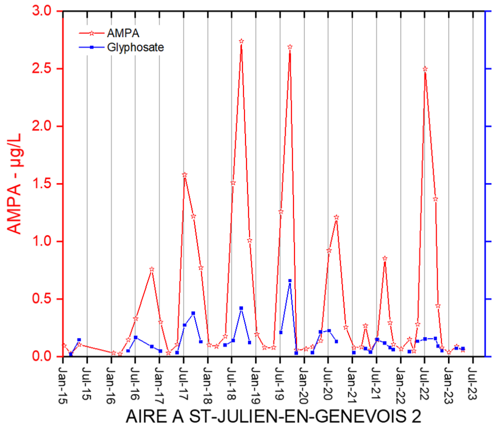
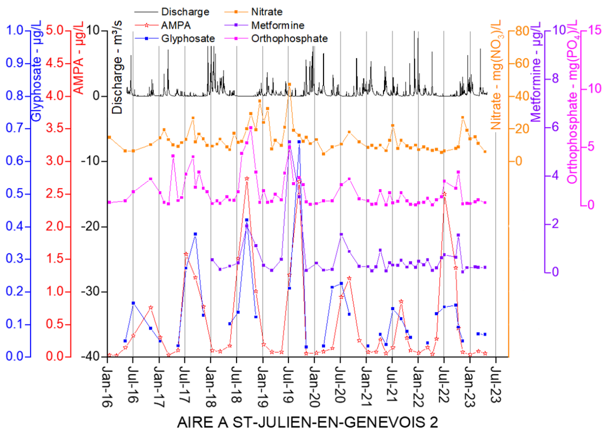
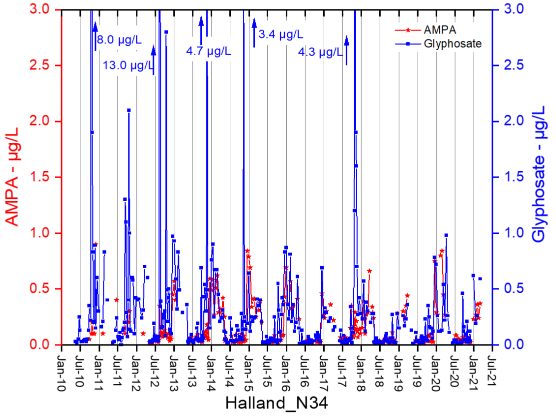
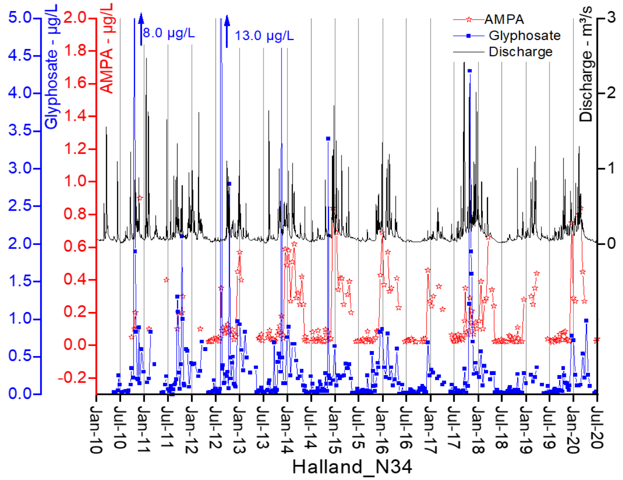
E28 **FR** **(84)**	**site:** 6117290_CORONNE A VALREAS 4 **catchment:** mixed urban and agricultural (large fields) **WWTP:** Station de VALRÉAS CHEF-LIEU (15,200 IE) with tributary close to sampling point			
E29 **FR** **(11)**	**site:** 6179500_AUDE A LA REDORTE 1 **catchment:** 3,267 km² (Puichéric); vineyards, small villages, city of Carcassonne **WWTP:** Station de ROQUECOURBE (150 IE), Station de CASTELNAU D'AUDE (350 IE) **discharge data:** Y142 2010 01 - L'Aude à Puichéric			

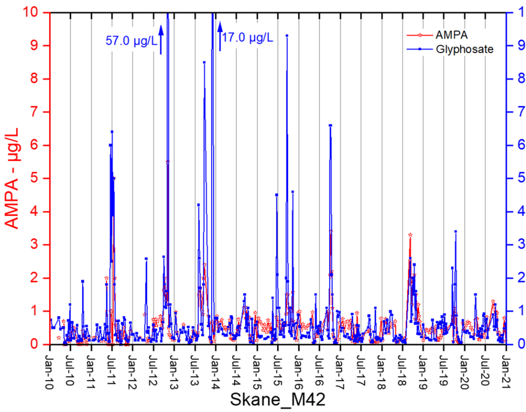
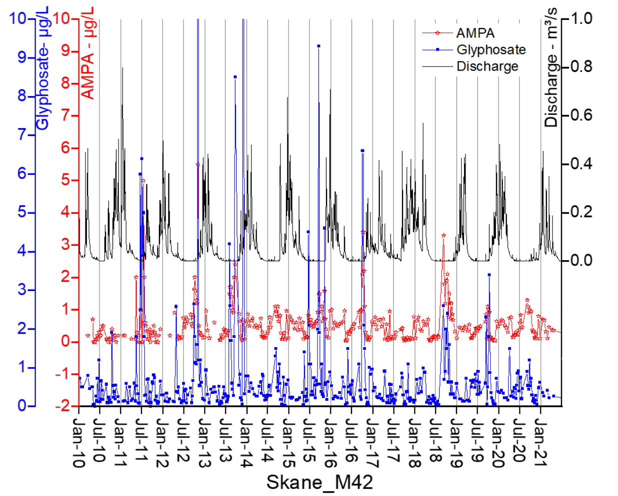
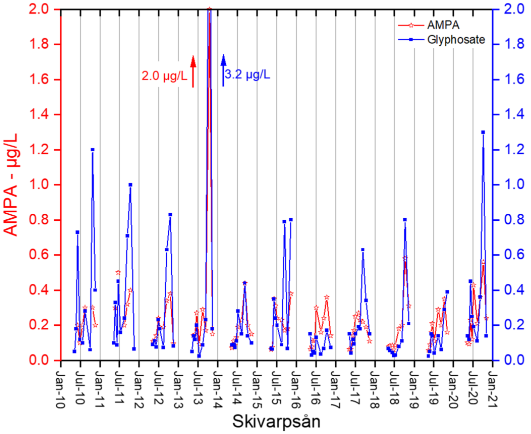
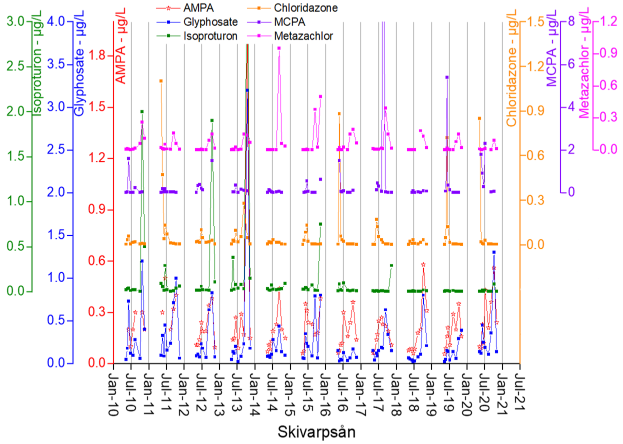
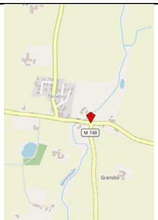
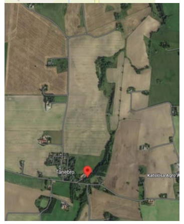
E30 **FR** **(11)**	**site:** 6180000_AUDE A MOUSSAN 1 downstream of site E29 **catchment:** 4,838 km²; agriculture (vineyards), some villages, city of Carcassonne **WWTP:** Station de MOUSSAN (1,200 IE) close to sampling point, Station de VAL DE CESSE (11,500 IE, tributary)	 6180000_AUDE A MOUSSAN 1	 6180000_AUDE A MOUSSAN 1	
E31 **FR** **(11)**	**site:** 6180900_AUDE A SALLES-D'AUDE downstream of site E30 **catchment:** 4,900 km² (Coursan); agriculture (vineyards), some villages, city of Carcassonne **WWTP:** Station de FLEURY-SALLES (7,600 IE), Station de COURSAN (4,600 IE)	 6180900_AUDE A SALLES-D'AUDE	 6180900_AUDE A SALLES-D'AUDE	

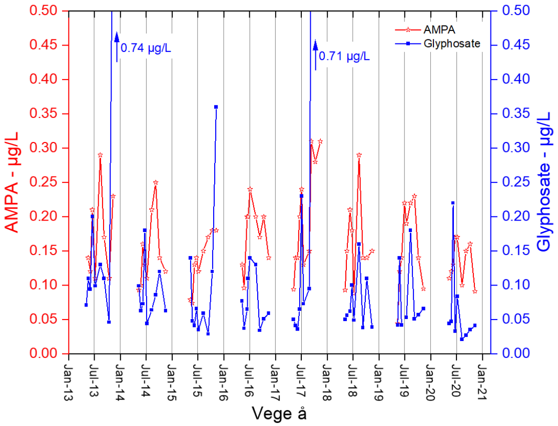
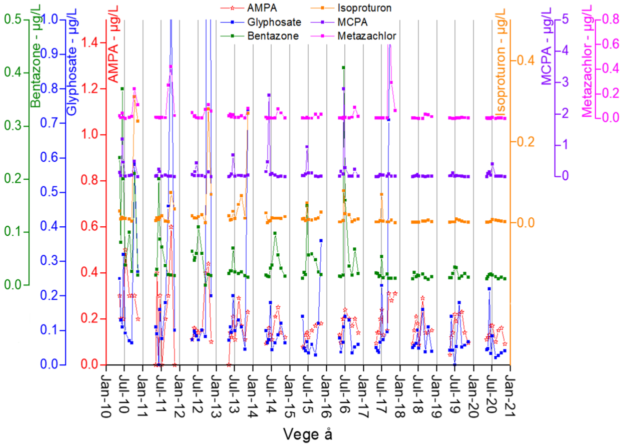
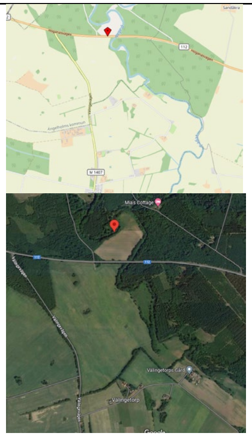
E32 **FR** **(30)**	**site:** 6193250_VISTRE DE LA FONTAINE A NIMES **catchment:** 41 km²; fully urban; spring in Nîmes, partially in canal from Roman times (used as old sewer system); few fields after leaving the city **WWTP:** no WWTP, but some houses connected to old sewer			
E33 **FR** **(30)**	**site:** 6193500_VISTRE À AUBORD **catchment:** 291 km² (Bernis); mixed agriculture (fields) and city of Nîmes **WWTP:** STEU de la commune de NIMES (220,000 IE), STEU de la commune de MARGUERITTES (11,000 IE) **discharge data:** Y351 4020 01 - Le Vieux Vistre à Bernis			

E34 FR (30)	**site:** 6193700_VISTRE A ST-LAURENT-D'AIGOUZE downstream of site E31 **catchment:** 490 km² (Cailar); mixed urban (Nîmes, 150,000 inh.; Vauvert, 12,000 inh.) and agriculture (grassland, fields, vineyards), swampland **WWTP:** Station de LE CAILAR (6,300 IE), Station de VAUVERT (28,400 IE), Station de CODOGNAN, (9,700 IE, tributary)	 6193700_VISTRE A ST-LAURENT-D'AIGOUZE	 6193700_VISTRE A ST-LAURENT-D'AIGOUZE	
35 FR (13)	**site:** 6195500_ARC A BERRE-L'ETANG **catchment:** 692 km²; agriculture with various crops incl. olives, large industrial site, large city (Aix en Provence) **WWTP:** Station de LA FARE LES OLIVIERS (7,800 IE), Station de COUDOUX-VENTABREN-VELAUX (17,500 IE). Station de AIX-OUEST (23,000 IE), Station de AIX EN PROVENCE LA PIOLINE (140,000 IE)	 6195500_ARC A BERRE-L'ETANG	 6195500_ARC A BERRE-L'ETANG	

E36 **FR** **(70)**	**site:** 6416300_DURGEON A VESOUL 3 **catchment:** ca. 200 km²; agricultural (grassland, some fields, forests), small villages **WWTP:** several smaller WWTP (<300 IE)			
E37 **FR** **(90)**	**site:** 6460950_DOUCE A BERMONT **catchment:** urban (e.g. Belfort (45,000 inh.)), fields, forest, grassland **WWTP:** Station de BAVILLIERS (8,700 IE)			

E38 **FR** **(74)**	**site:** 6999125_AIRE A ST-JULIEN-EN-GENEVOIS 2 **catchment:** 40 km²; agriculture, urban (St. Julien-en-Genevois and smaller villages) **WWTP:** Station de NEYDENS (16,500 IE) **discharge data:** V024 5610 01 - L'Aire à Saint-Julien-en-Genevois [Thairy] low discharge and strong WWTP influence			
	Sweden			
E39 **SE**	**site:** N34, field site in Halland **catchment:** 14 km² with 85% farmland from Swedish monitoring program[253] **WWTP:** no WWTP, 19 people/km² connected to private sewers (https://jordbruksvatten.slu.se/pesticider_start.cfm, 14/10/2023)			

E40 SE	**site:** M42 fields in Skåne **catchment:** 8 km² with 92% farmland from Swedish monitoring program[253] **WWTP:** no WWTP; 10 people/km² connected to private sewers ([https://jordbruksvatten.slu.se/pesticider_start.cfm, 14/10/2023](https://jordbruksvatten.slu.se/pesticider_start.cfm))			
E41 SE	**site:** Skivarpsån **catchment:** rural area, Skurup and Rydsgård as smaller cities **WWTP:** no WWTP only summer data available			 

E42 **SE**	**site:** Vege å **catchment:** mixed agricultural land and several cities **WWTP:** Åstorp (13,000 IE), Ekebo (11,000 IE), Kågeröd (2,500 IE) only summer data available			

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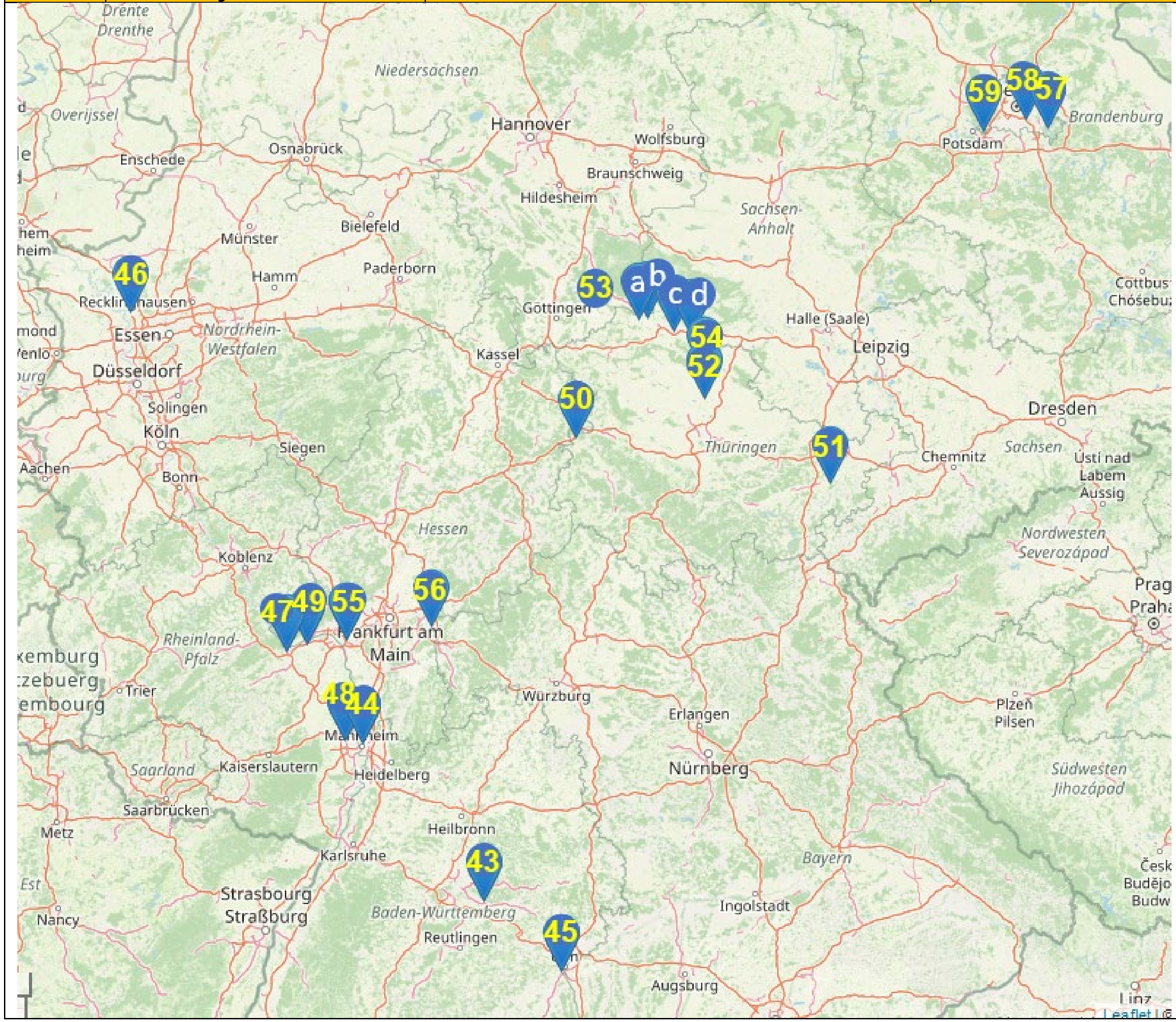
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Wolfsburg
Brandenburg
Potsdam
Enschede
Osnabrück
Braunschweig
Hildesheim
Sachsen-
Anhalt
Bielefeld
Münster
Paderborn
Hamm
Cottbus
Chóśebuz
Recklinghausen
Göttingen
Halle (Saale)
Essen
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Leipzig
Kassel
Düsseldorf
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Dresden
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Pfalz
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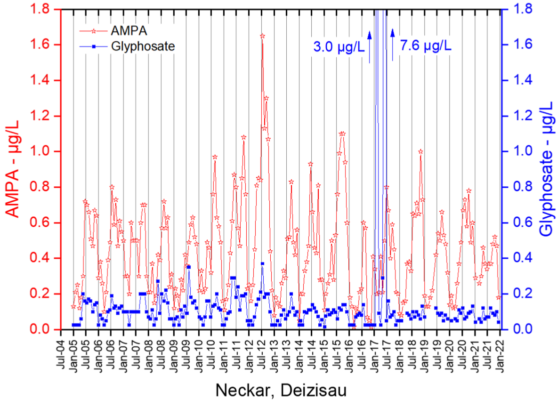
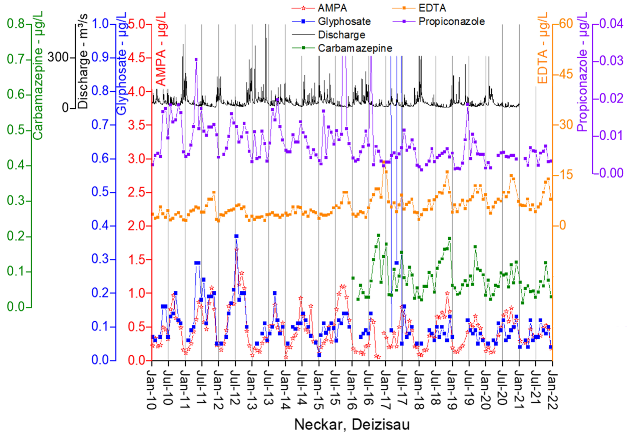
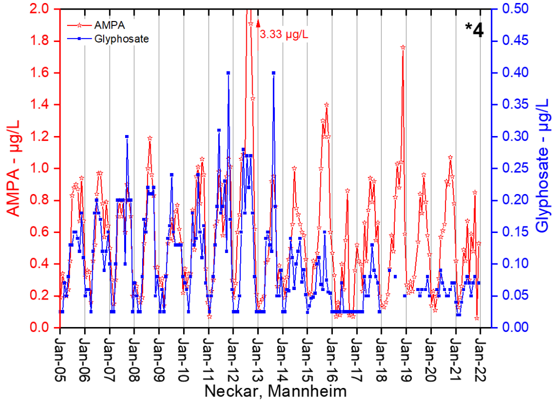
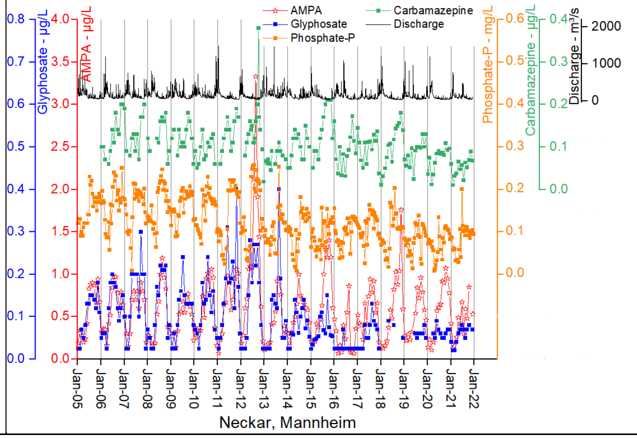
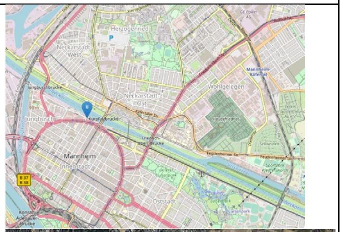
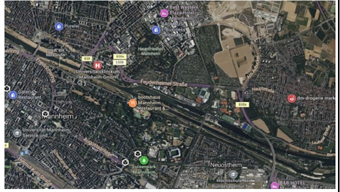
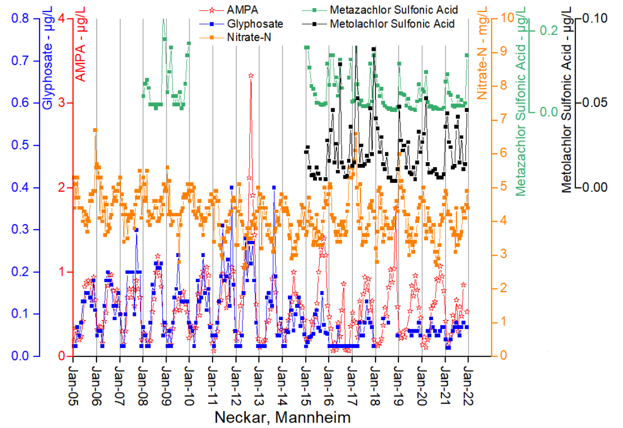
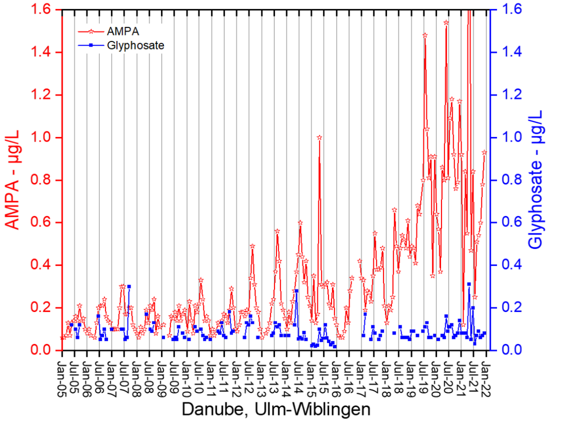
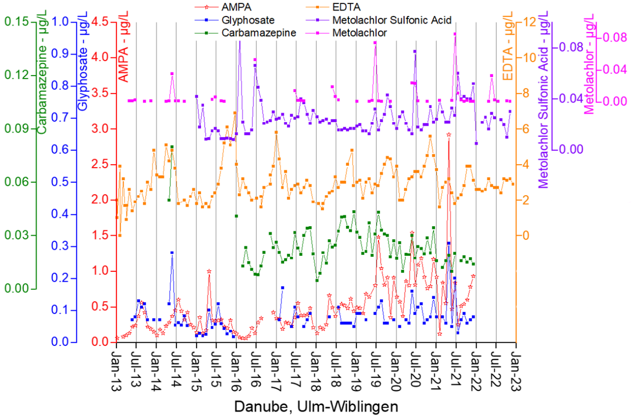
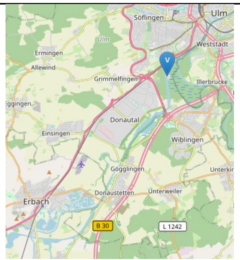
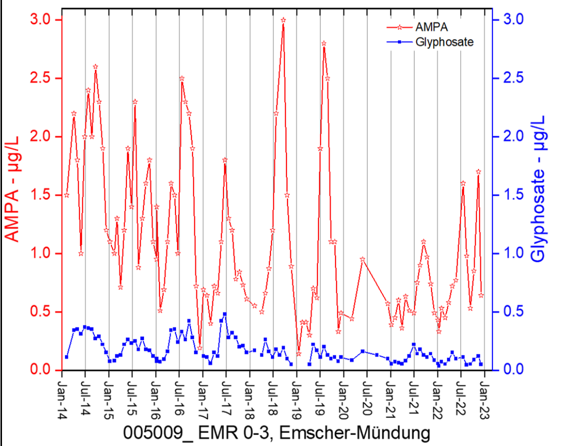
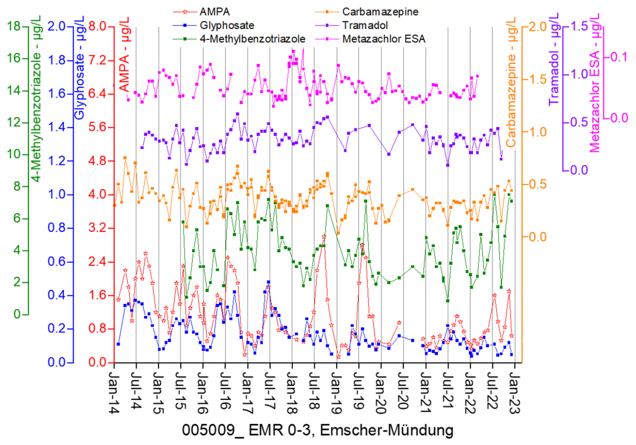
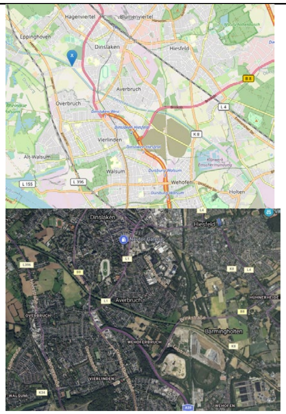
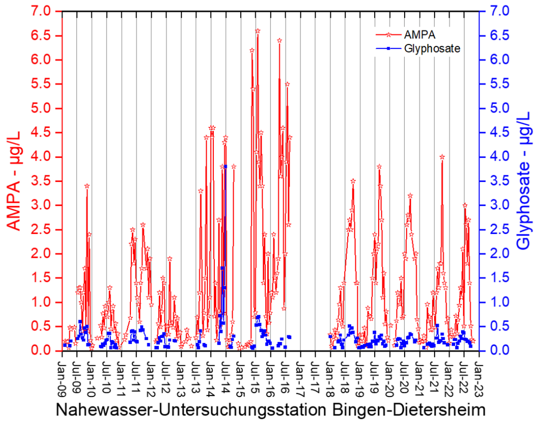
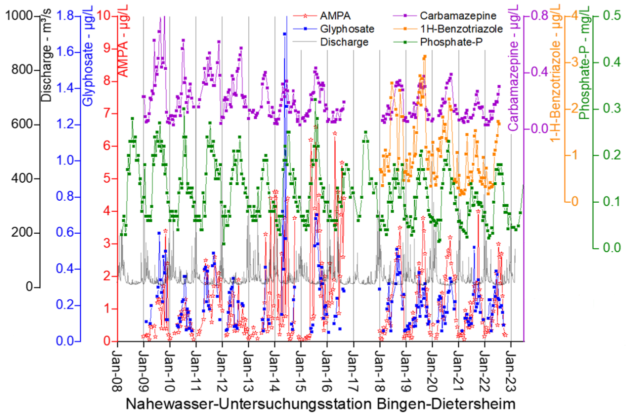
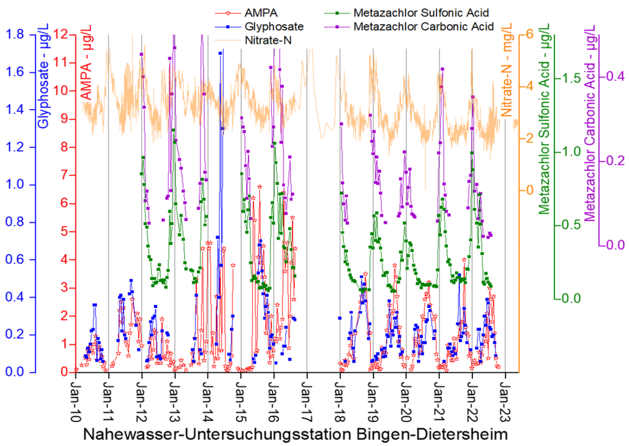
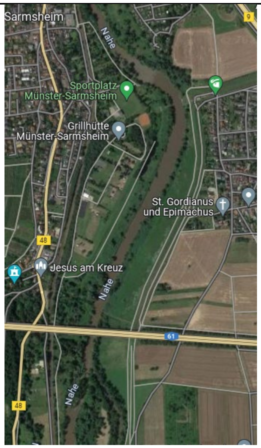
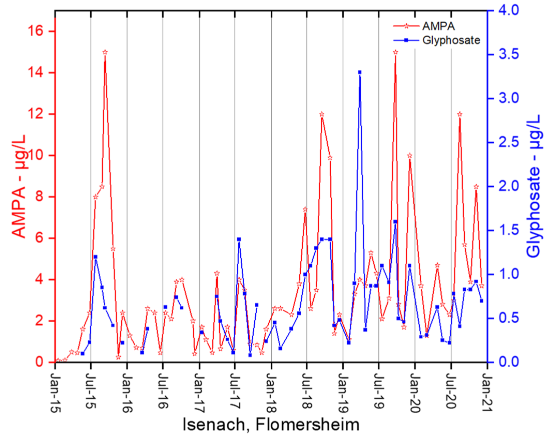
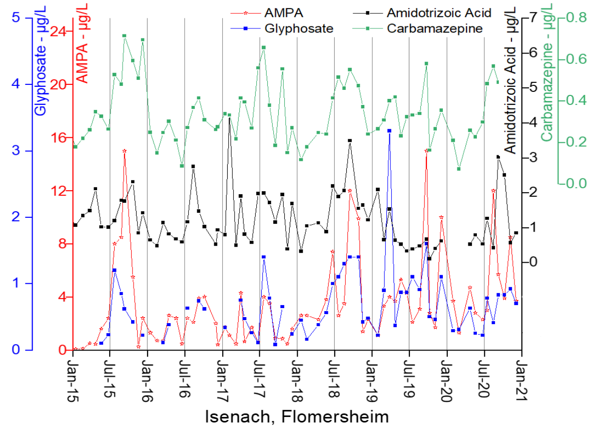
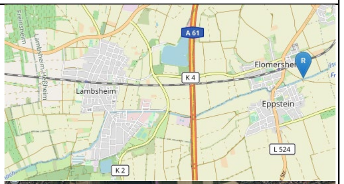
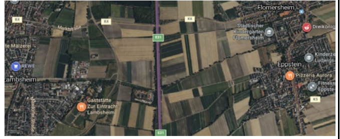
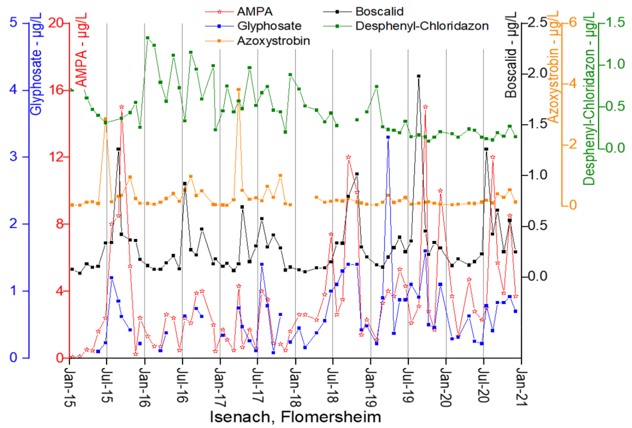
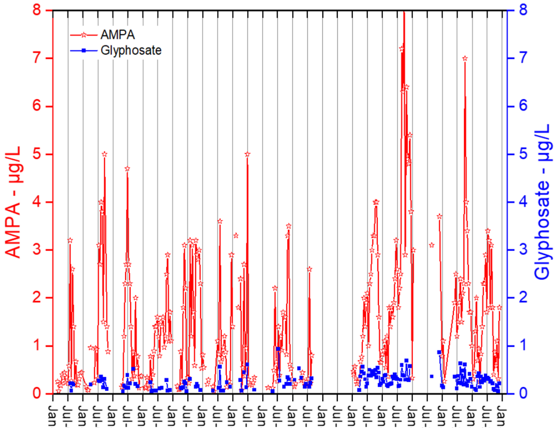
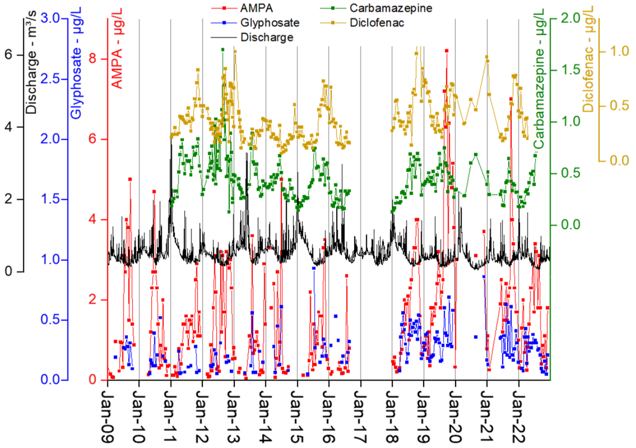
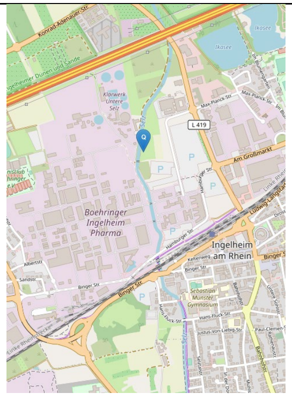
Saarbrücken
Metz
Heilbronn
Karlsruhe
Bayern
Est
Strasbourg
Straßburg
Baden-Württemberg
Ingolstadt
Nancy
Reutlingen
Augsburg
Linz
Leaflet

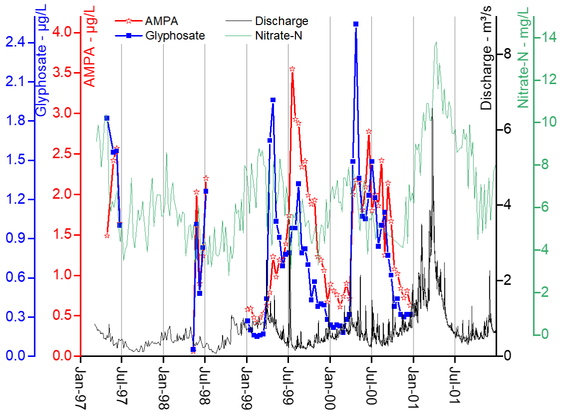
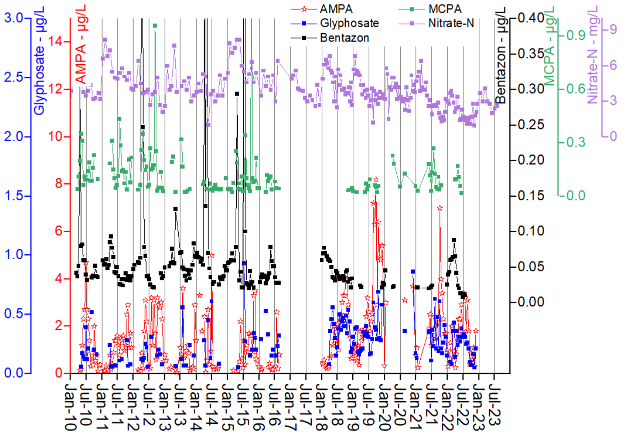
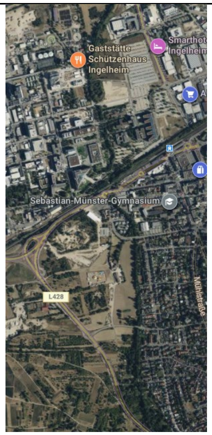
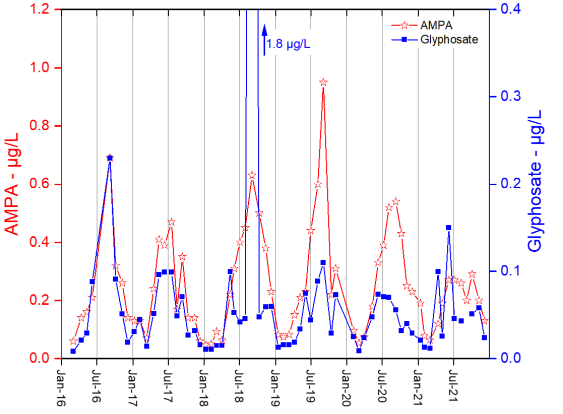
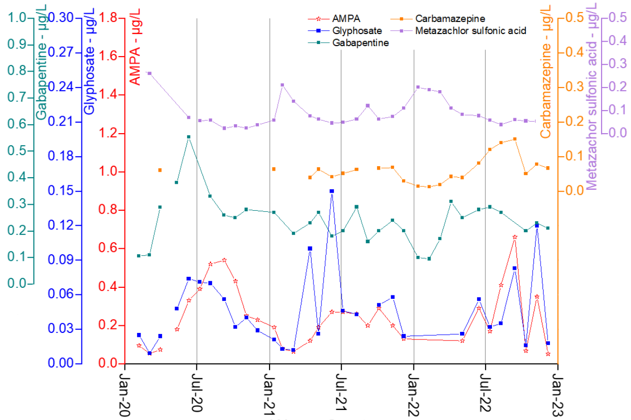
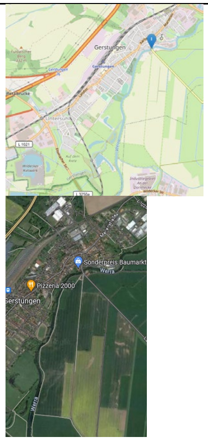
E43 **DE** **(BW)**	**site:** CYY200_Neckar, Deizisau **catchment:** 3,995 km² (Plochingen); urban around sampling site, agricultural sections, cities Reutlingen (120,000 inh.),Tübingen (93,000 inh.) **WWTP:** Wernau (30,000 IE), Reichenbach a.d. Fils (31,000 IE, tributary), Wendlingen (170,000 IE), Nürtingen (75,000 IE), Neckartenzlingen (19,000 IE), Tübingen (137,000 IE)			
E44 **DE** **(BW)**	**site:** CYY003_Neckar, Mannheim **catchment:** 13,900 km²; mixed urban and agriculture **WWTP:** closest large WWTPs: Neckarhausen (80,000 IE), Heidelberg (360,000 IE) (on total ca. 5.5 Mio inhabitants)			

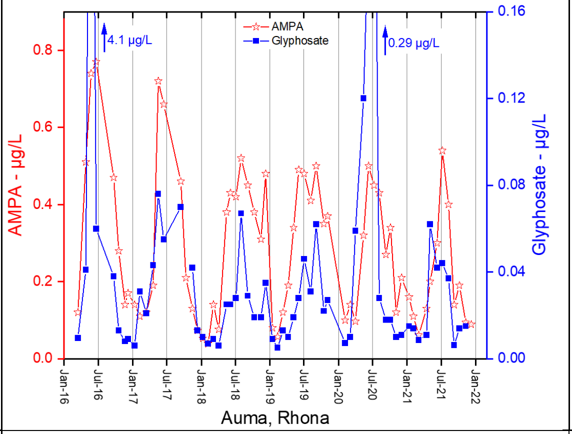
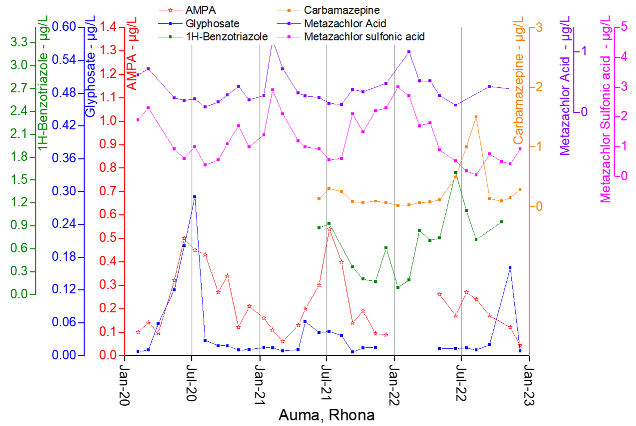
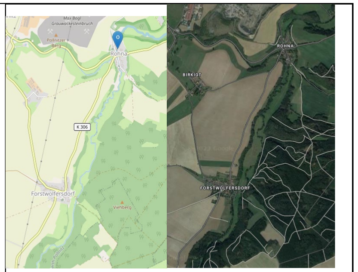
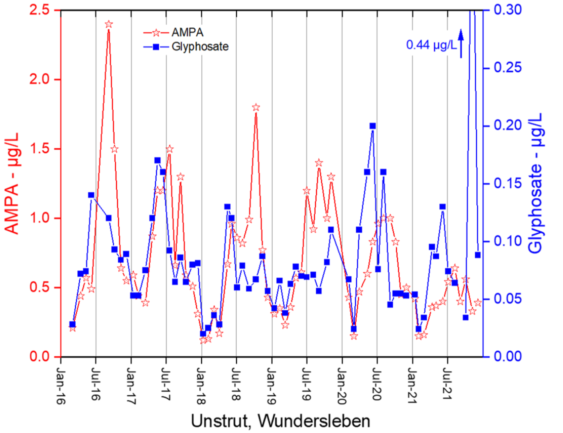
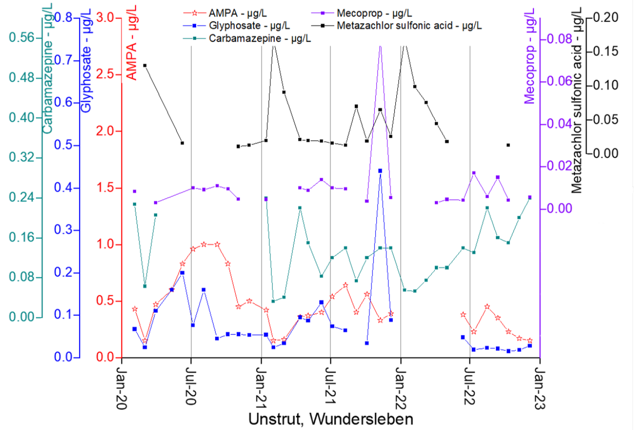
				
E45 **DE** **(BW)**	**site:** CQQ803_Danube, Ulm-Wiblingen **catchment:** 5,000 km²; agriculture with smaller cities and villages **WWTP:** Erbach (20,000 IE), Öpfingen (8,000 IE)			 

E46 **DE** **(Nrw)**	**site:** 005009_ EMR 0-3, Emscher (mouth) **catchment:** 794 km^2; dominantly urban, few fields **WWTP:** Emscherkläranlage (1.4 Mio IE) close to sampling site, Bottrop (1.3 Mio IE), Dortmund-Deusen (700,000 IE)			
E47 **DE** **(Rhp)**	**site:** 2540502500_Nahewasser-Untersuchungsstation Bingen-Dietersheim 14-day composite samples **catchment:** 4,039 km^2; mixed urban and agriculture with city Bad Kreuznach **WWTP:** Nahetal (35,000 IE, tributary), Langenlons-heim (30,000 IE), Gulden-bachtal (22,000 IE, tributary), Bad Kreuznach (110,000 IE)			

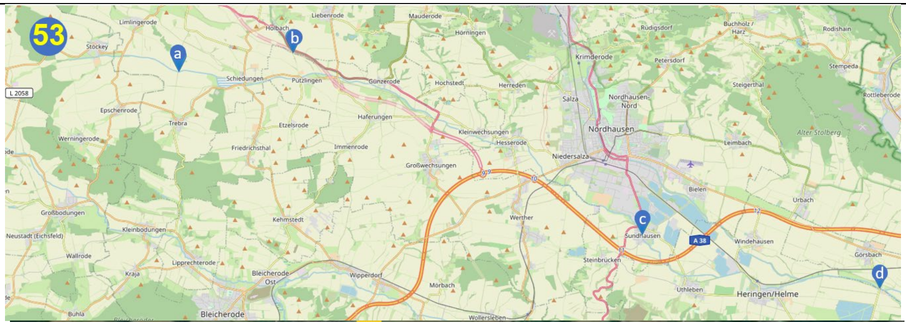
			 Nahewasser-Untersuchungsstation Bingen-Dietersheim	
E48 **DE** **(Rhp)**	**site:** 2391513000_Isenach, Pegel Flomersheim **catchment:** 279 km²; agricultural (wine, legume) and villages, city of Bad Dürkheim **WWTP:** Lambsheim (25,000 IE), Weisenheim am Sand (24,000 IE, tributary)	 Isenach, Flomersheim	 Isenach, Flomersheim	 

			 Isenach, Flomersheim	
E49 **DE** **(Rhp)**	**site:** 2529522200_Selz, Ingelheim **catchment:** 363 km² (Oberingelheim); mixed urban and agriculture (wine-growing), large pharmaceutical company **WWTP:** Saulheim (30,000 IE, tributary), Hahnheim (18,000 IE), Bechtolsheim (18,000 IE), Alzey (36,000 IE)	 Selz, Ingelheim	 Selz, Ingelheim	

		 Selz, Ingelheim	 Selz, Ingelheim	
E50 DE (Thu)	**site:** 2371_Werra at Gerstungen **catchment:** 3,039 km²; mixed urban and agriculture, villages along the river **WWTP:** Wildeck/Obersuhl (5,200 IE, tributary), Heringen (12,000 IE), Philippsthal (8,000 IE), Vache (7,500 IE), …	 Werra, Gerstungen	 Werra, Gerstungen	

E51 DE (Thu)	**site:** 58681_Auma at Rhona **catchment:** 136 km² (Eisenhammer); forest, fields, some small villages **WWTP:** Niederpöllnitz (2,500 IE), Auma (3,100 IE)			
E52 DE (Thu)	**site:** 2167_Unstrut at Wundersleben **catchment:** 2,049 km² (Straußleben); agricultural with smaller villages, Bad Langesalza (17,000 inh.), flood control reservoir close to sampling site **WWTP:** Straußfurt (close to sampling point, 6,000 IE), Gebesee (3,000 IE, tributary), Herbsleben (4,800 IE), Bad Langensalza (33,000 IE)			

E53 a-d DE (Thu)	a	N° 2114	Helme, Schiedunger Teiche oh (catchment ca. 25 km²); WWTP Stöckey (400 inh.)
	b	N° 2113	Ichte, Pützlingen (above confluence with Helme, catchment ca. 50 km²); small WWTPs (Mackenrode (550 inh.), Holbach 200 inh.)
	c	N° 2134	Helme, Sundhausen (catchment 201 km²); several small WWTPs with several thousand inh.
	d	N° 2135	Helme, Aumühle (catchment ca. 550 km²); WWTP Sundhausen (100,000 IE)

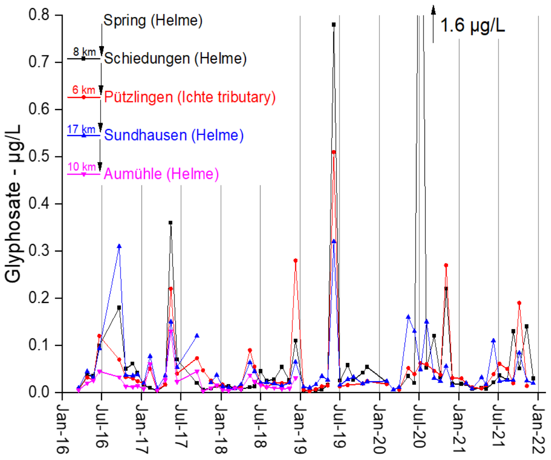


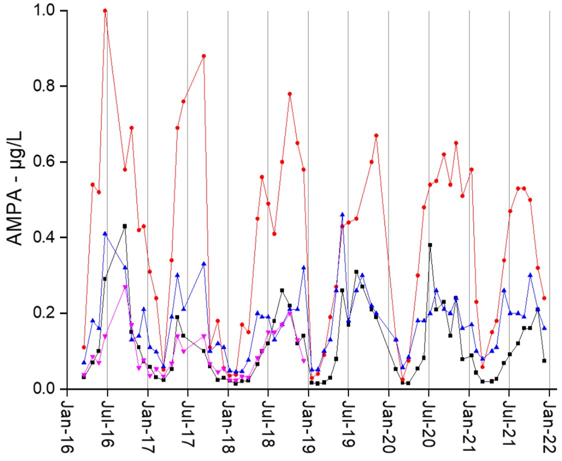
site: Helme, Thüringen

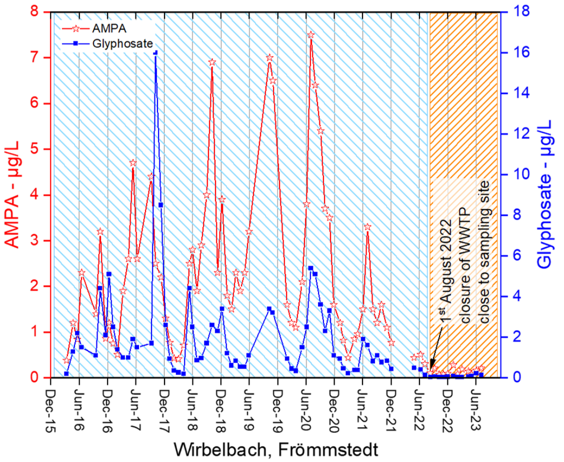
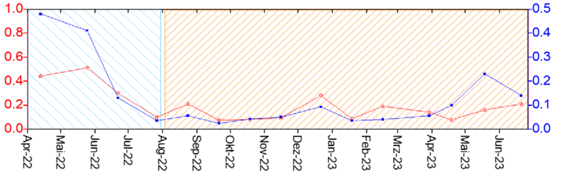
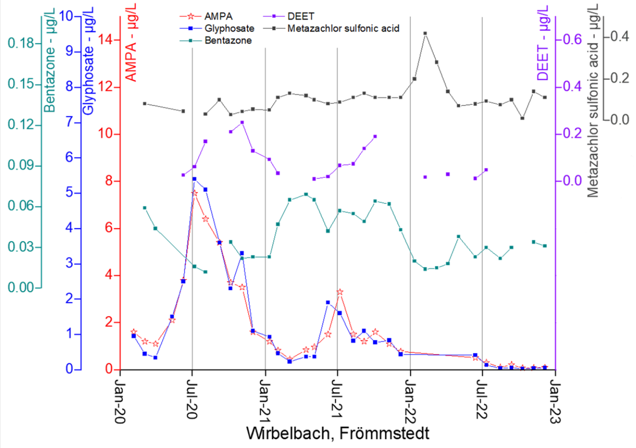
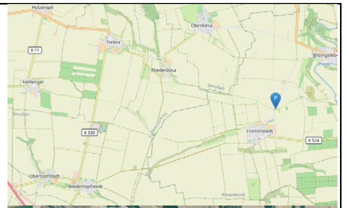
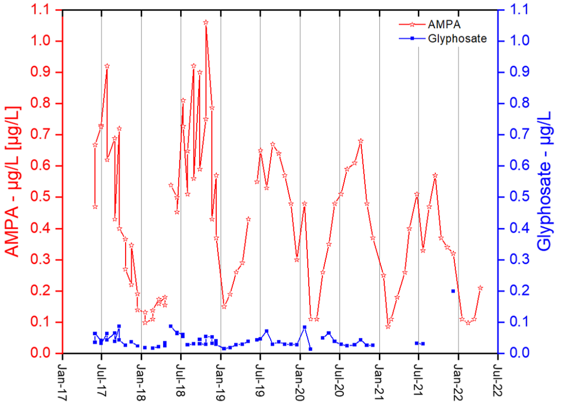
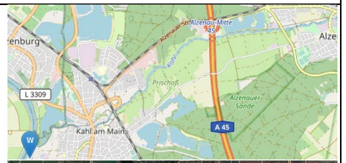
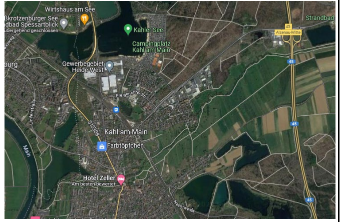
sampling ca. 8 km after spring

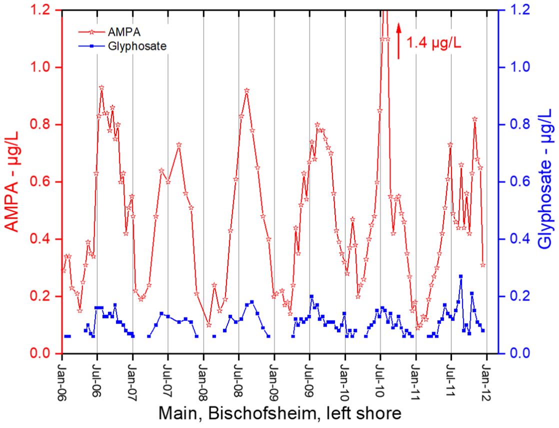
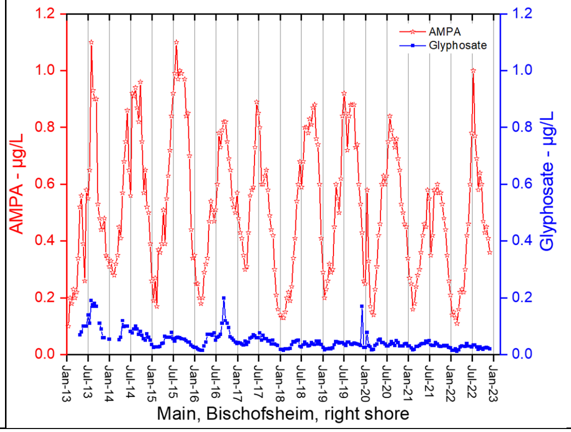
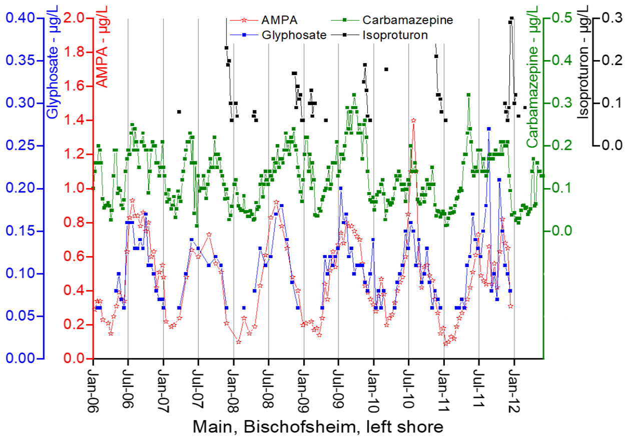
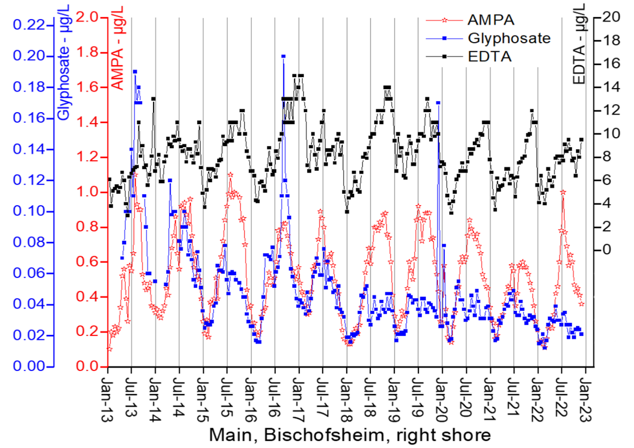
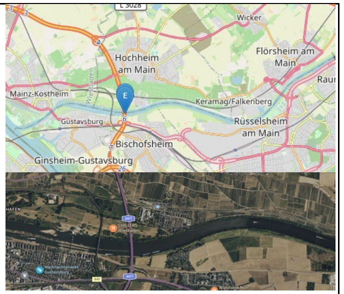
catchment: agriculture with large fields; city of Nordhausen and Sundhausen via tributary

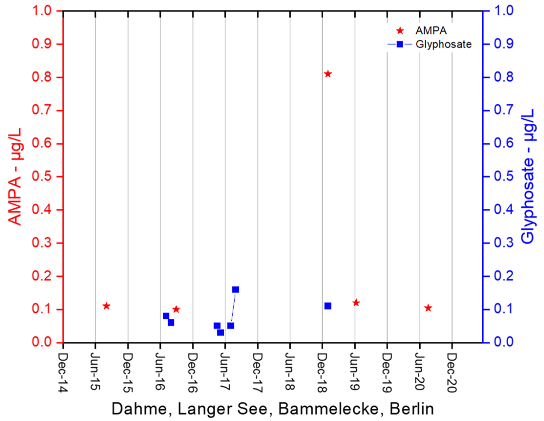
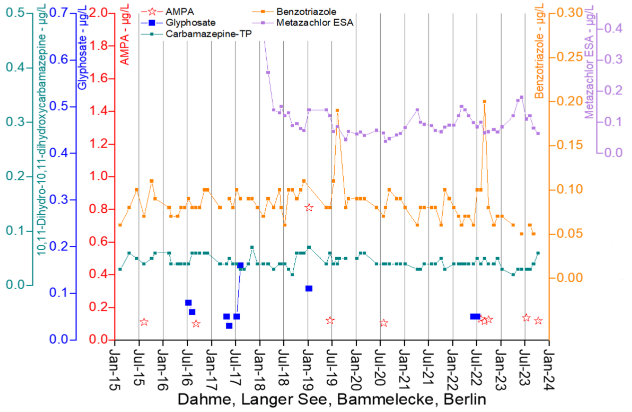
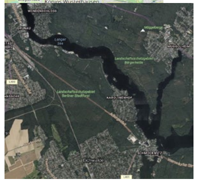
WWTP: Stöckey (400 inhabitants), Nordhausen (100,000 IE, tributary), Aumühle (10,000 IE), large number of small WWTP in Pützlingen, Haferungen, Großwechsungen, Kleinwerther, Großwerther

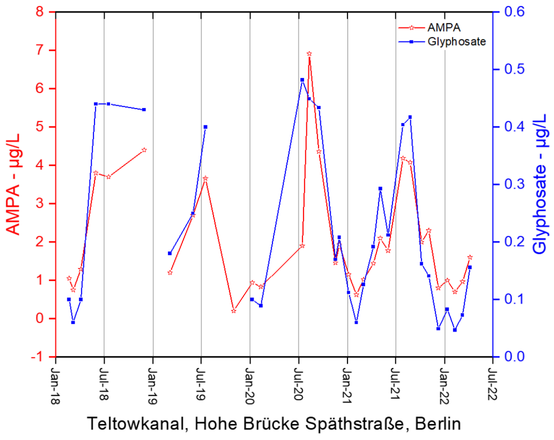
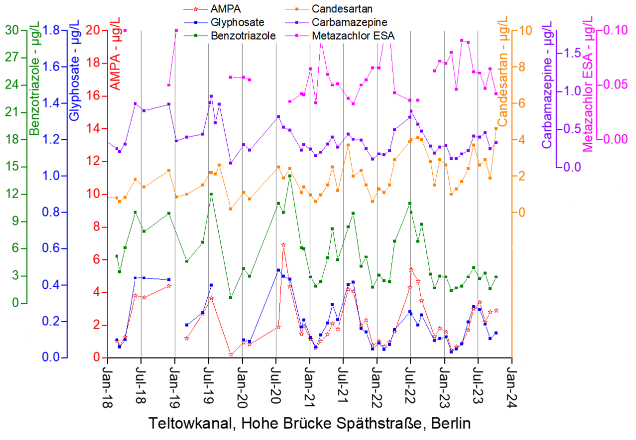
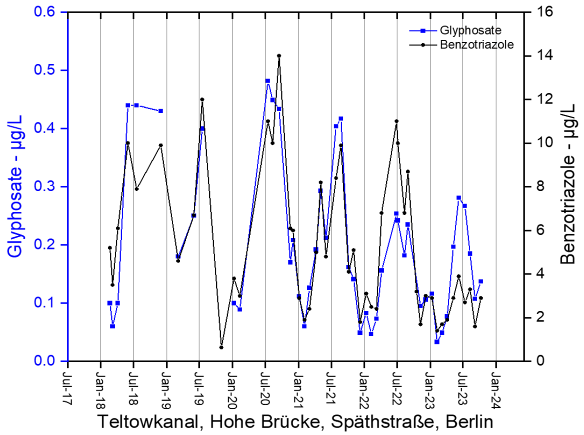
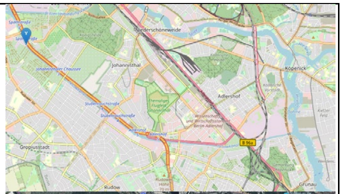


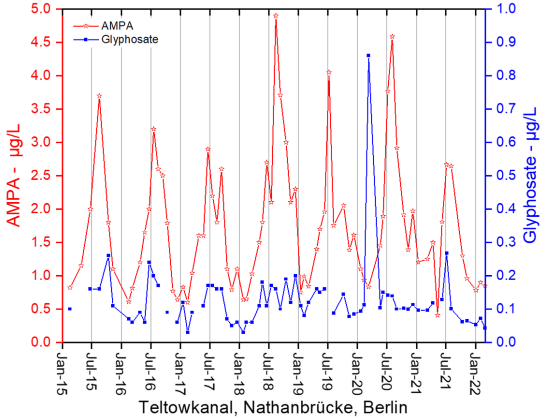
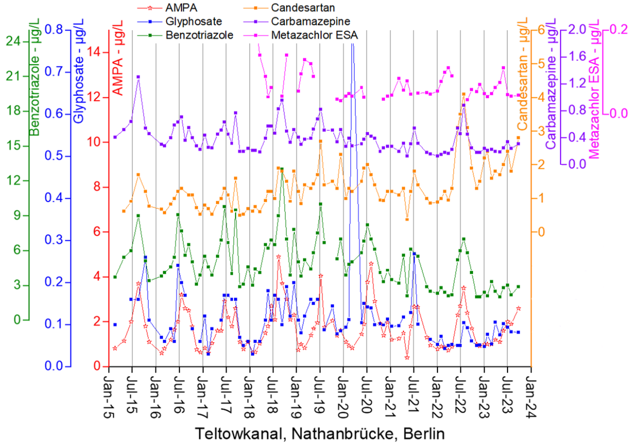


E54 **DE** **(Thu)**	**site:** 3721_Wirbelbach at Frömmstedt, sampling ca. 15 km after spring **catchment:** ca. 35 km²; agricultural area with large fields (ca. 60 km from site 53), small villages **WWTP:** private sewer from baking company in Molchbrongraben, WWTP Frömmstedt (500 inh., large baking facility with 290 IE), private sewers sewage treatment plant conversion at 1st August 2022 to Kindelbrück	 Wirbelbach, Frömmstedt 	 Wirbelbach, Frömmstedt	 
E55 **DE** **(Bay)**	**site:** 24078008_Kahl a. Main Messstation / Main **catchment:** 23,374 km² (Krotzenburg); mixed urban and agriculture **WWTP:** Alzenau (25,000 IE), Mömbris OT Brücken (40,000 IE)	 Main, Kahl a. Main Messstation / Main	no further data available	 

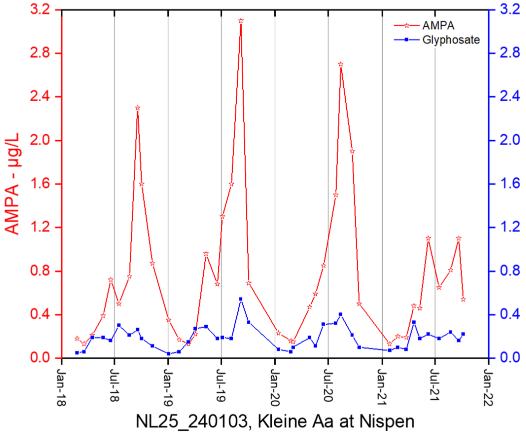
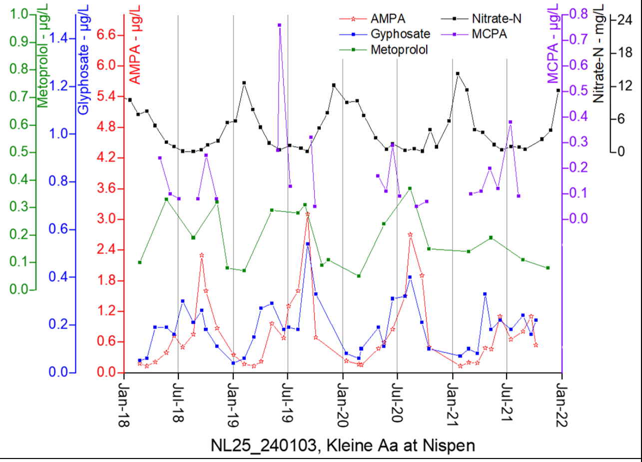
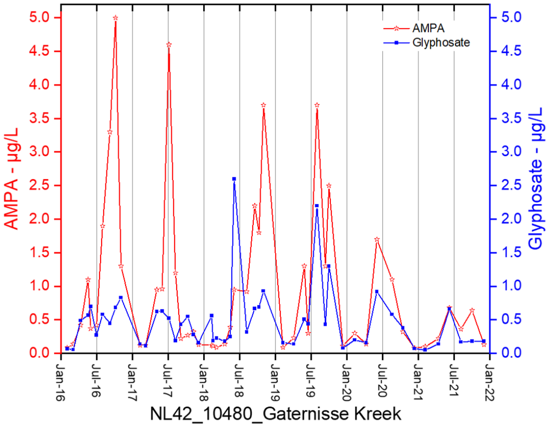
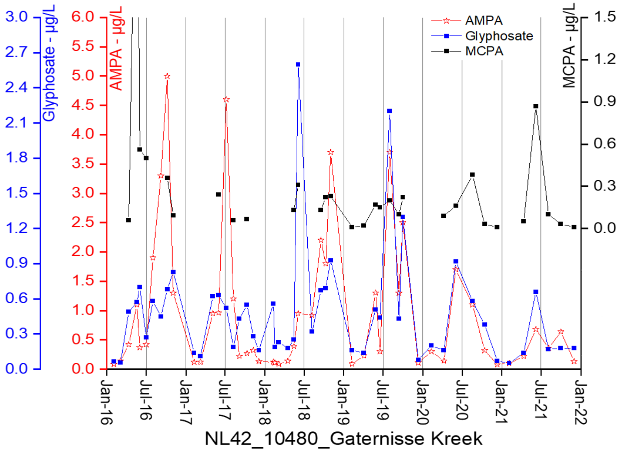
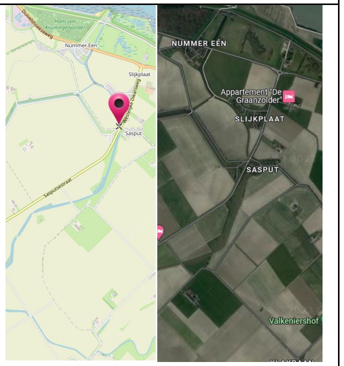
E56 **DE** **(Hes)**	**site:** Main, Bischofsheim **catchment:** 27,142 km^2 (Raunheim); urban and agriculture sampling at both banks independently (different wastewater input assumed) right bank: 14-day composite sample; left bank: 7-day composite sample **WWTP:** Hochheim (35,000 IE), Flörsheim (90,000 IE), Raunheim (98,000 IE), Hattersheim (6,500 IE), Frankfurt a. Main (470,000 IE)	 Main, Bischofsheim, left shore  Main, Bischofsheim, right shore	 Main, Bischofsheim, left shore  Main, Bischofsheim, right shore	

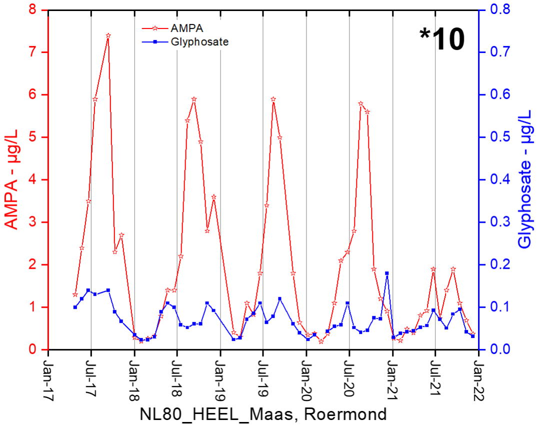
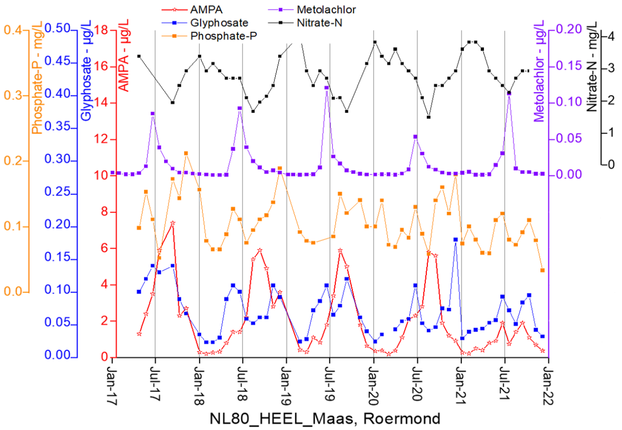
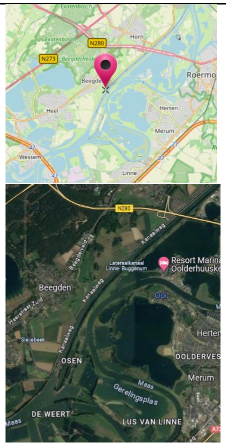
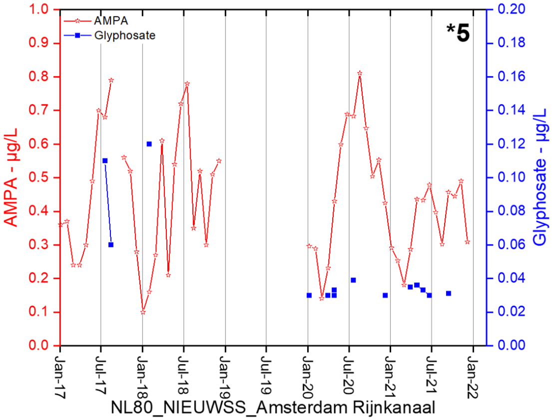
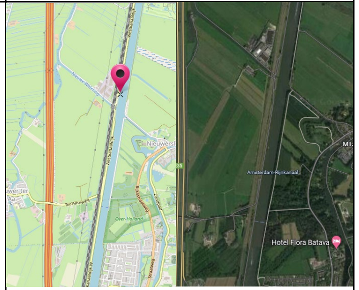
E57 **DE** **(Ber)**	**site:** Dahme, Langer See, Bammelecke **catchment:** 1,890 km²; urban and forests (partially natural reserve) **WWTP:** no WWTP, but WWTP influence via Dahme-Umflutkanal			 

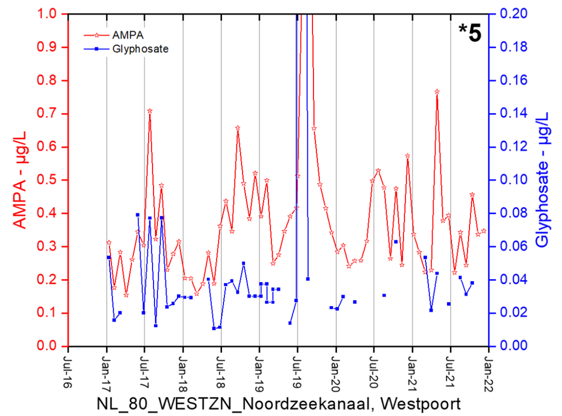
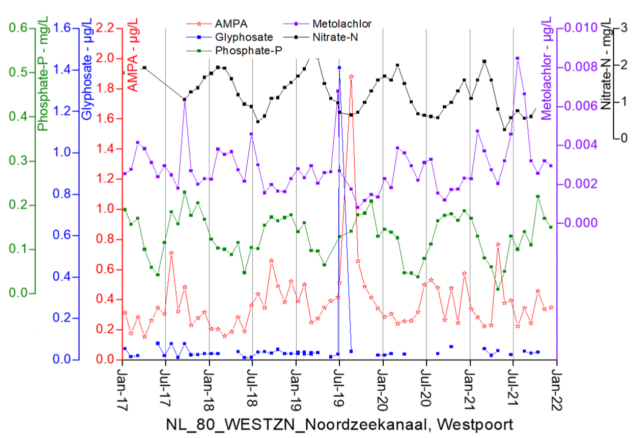
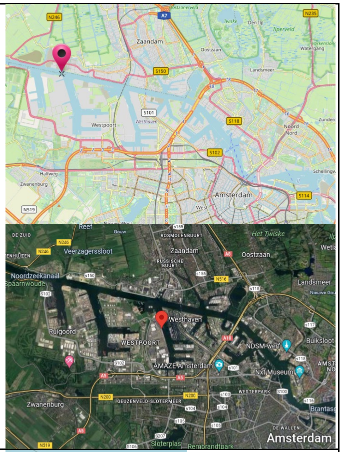
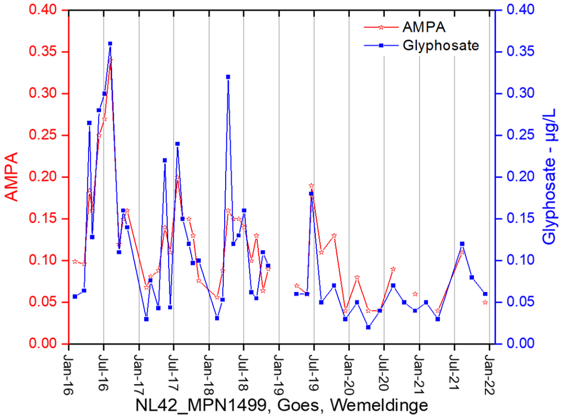
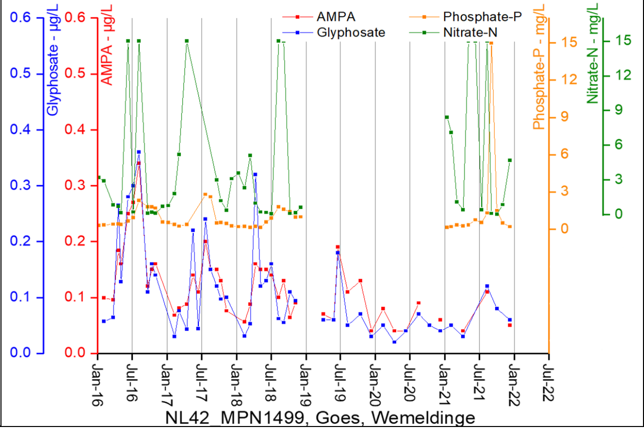
E58 **DE** **(Ber)**	**site:** Teltowkanal_Höhe Brücke Späthstraße fully urban, branch of Dahme (site E57) **catchment:** urban, branch of E57 **WWTP:** Waßmannsdorf (1.3 Mio IE)		 	 

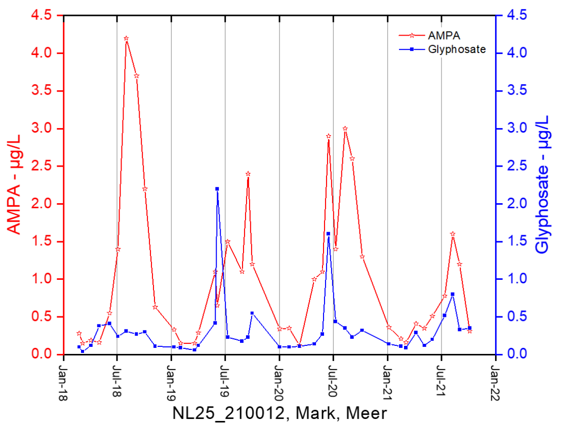
E59 **DE** **(Ber)**	**site:** Teltowkanal_ Nathanbrücke (LAWA) **catchment:** urban, forest downstream of site E58 **WWTP:** Waßmannsdorf (1.3 Mio IE), Stahndsorf (320,000 IE)			

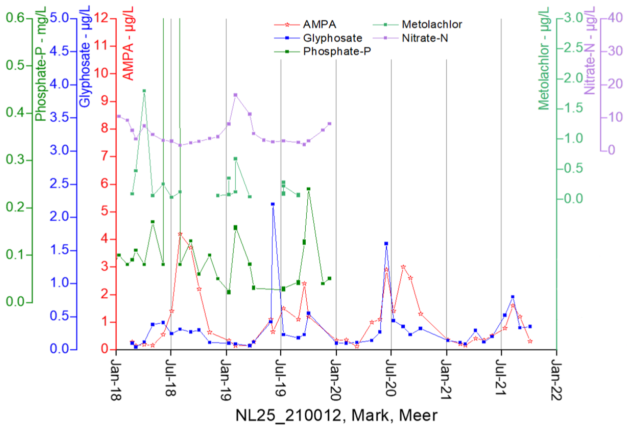
	The Netherlands			
				

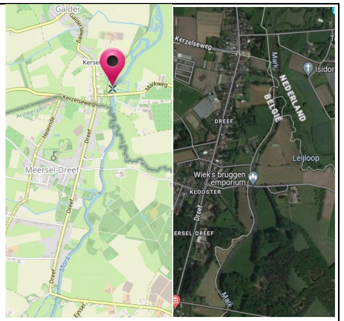
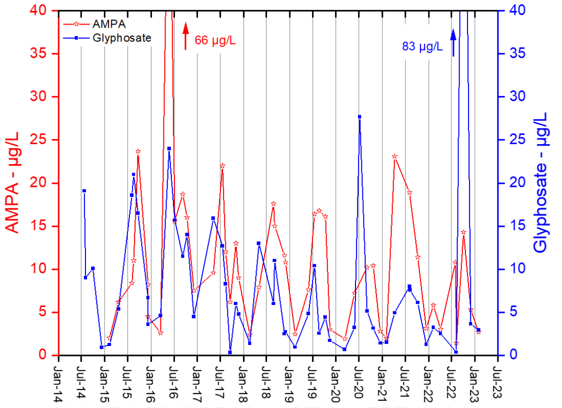
E60 NL	**site:** NL25_240103, Kleine Aa at Nispen **catchment:** agriculture and urban (Essen (BE) 19500 inh.) **WWTP:** Essen (14,000 IE), Kalmthout (28,000 IE)			
E61 NL	**site:** NL42_10480_Gaternisse Kreek **catchment:** agriculture, village Sasput **WWTP:** no WWTP, eventually private sewers			

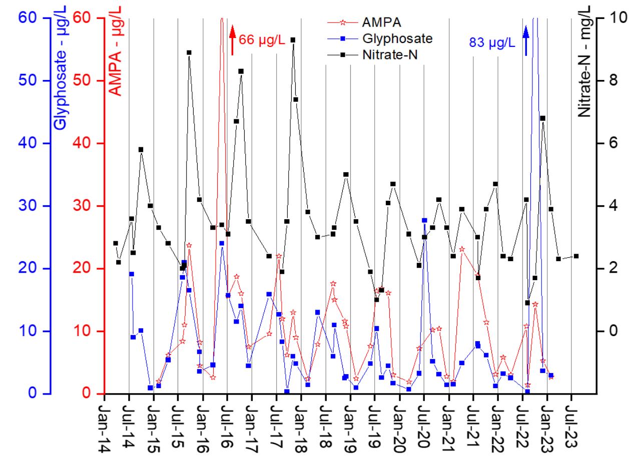
E62 **NL**	**site:** NL80_HEEL_Maas at Roermond sampling near drinking water abstraction site Heel **catchment:** mixed urban and agriculture, several water gates along river **WWTP:** several WWTP along Maas and tributaries, e.g. Susteren (300,000 IE), Stein (40,000 IE)			
E63 **NL**	**site:** NL80_NIEUWSS_Amsterdam Rijnkanaal **catchment:** mixed, strong urban influence from Utrecht **WWTP:** Breukelen (35,000 IE), Maarssen (tributary, 25,000 IE), Houten (90,000 IE), Leidsche Rijn (100,000 IE), Wijk bij Duurstede (30,000 IE)		other substances without suitable time series (mostly < LOD)	

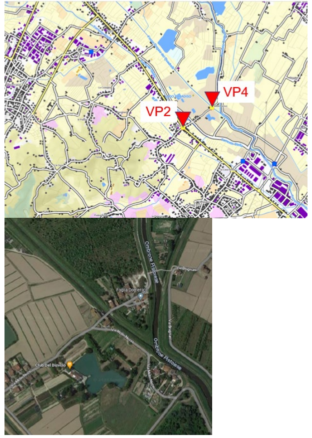
E64 **NL**	**site:** NL_80_WESTZN_Noord-zeekanaal at Westpoort **catchment:** urban and strongly drained fields **WWTP:** Westpoort (500,000 IE), Amsterdam West (1 Mio IE)			
E65 **NL**	**site:** NL42_MPN1499 Goes at Wemeldinge at pumping station to the ocean **catchment:** agriculture **WWTP:** no WWTP			

<table>
<tr><td>E66
NL</td><td>site: NL25_210012, Mark at Meer
catchment: agriculture and urban (Hoogstraten with 22,000 inh,), many drainage trenches
WWTP: Hoogstraten (26,000 IE)</td><td>

NL25_210012, Mark, Meer</td><td>

NL25_210012, Mark, Meer</td><td></td></tr>
<tr><td></td><td colspan="4">Italy</td></tr>
<tr><td>E67
IT
(Tos)</td><td>site: MAS-VP2_FOSSO DOGAIA DEI QUADRELLI at Catena
catchment: drainage trench in mixed agricultural and urban area
WWTP: Calice with almost 200,000 IE</td><td>

MAS-VP2_FOSSO DOGAIA DEI QUADRELLI, Catena</td><td>

MAS-VP2_FOSSO DOGAIA DEI QUADRELLI at Catena</td><td>
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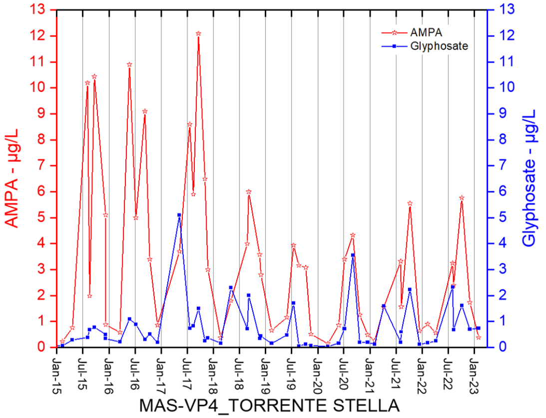
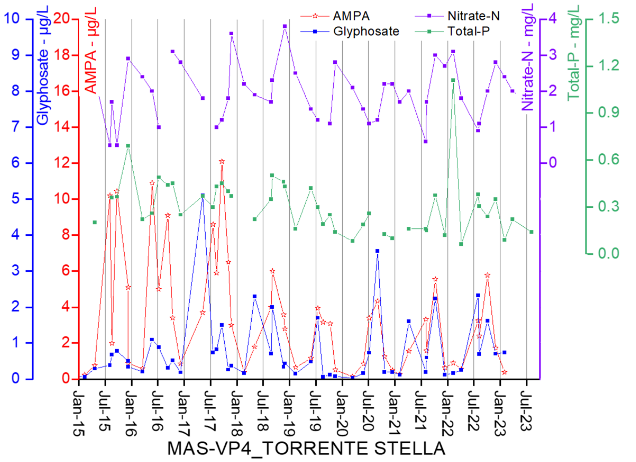
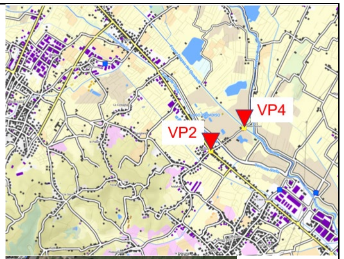
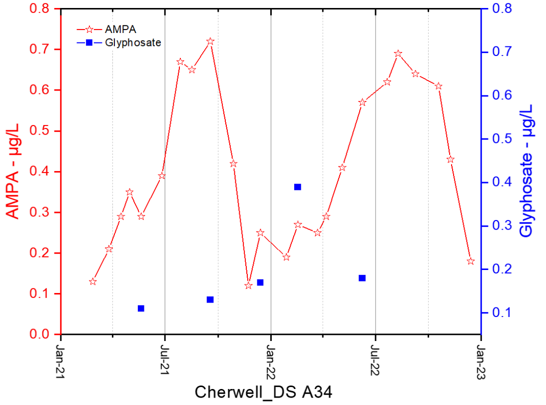
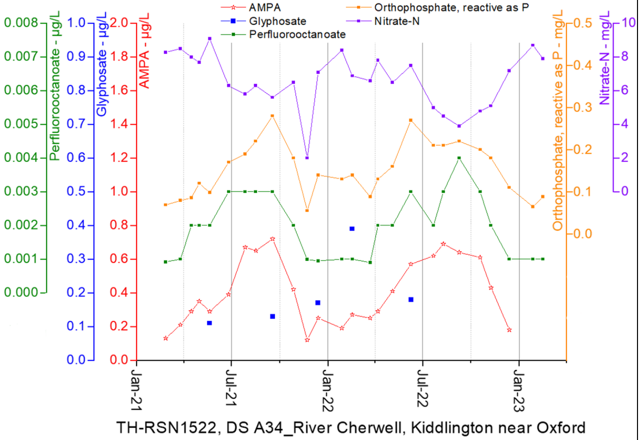
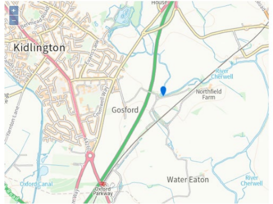
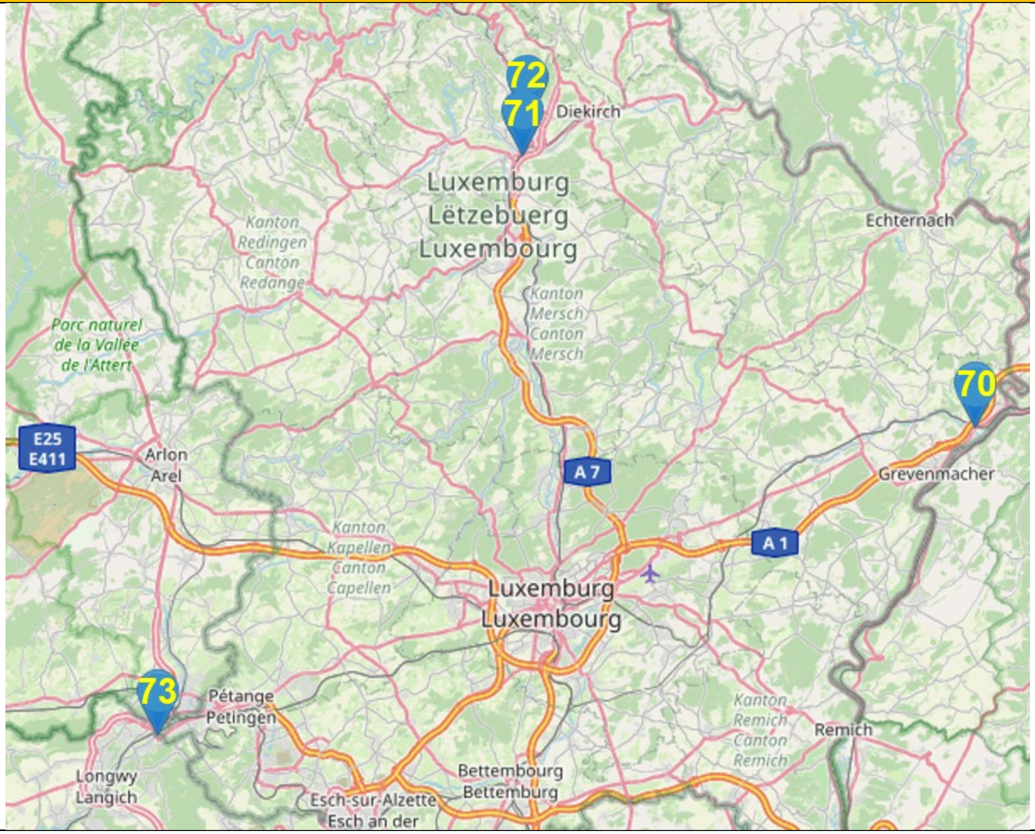
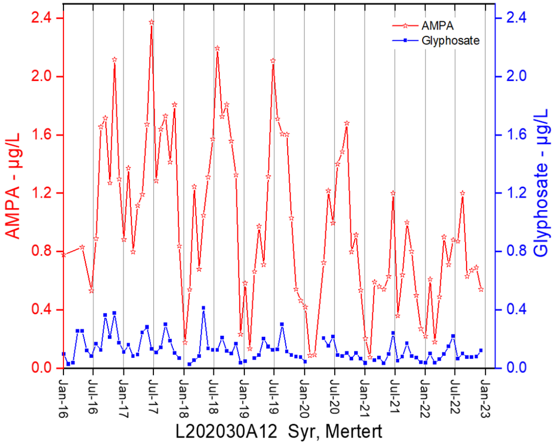
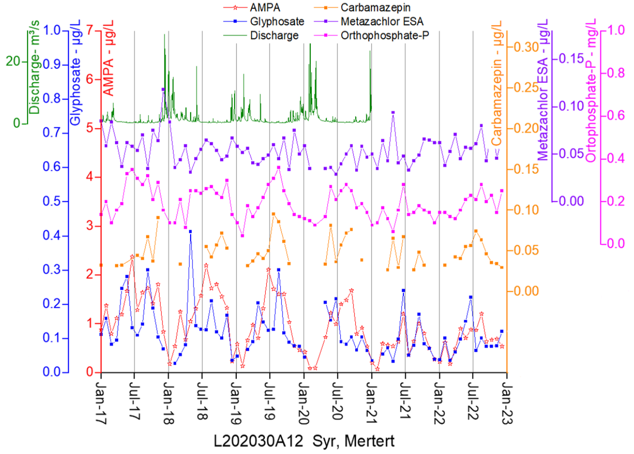
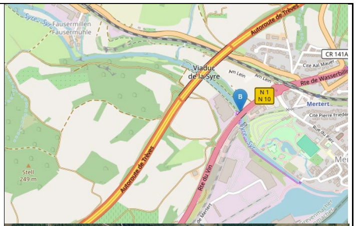
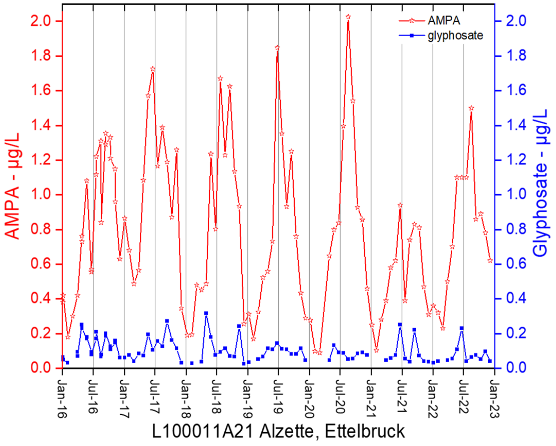
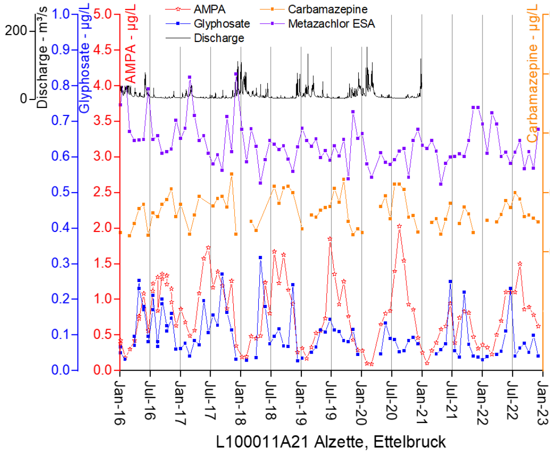
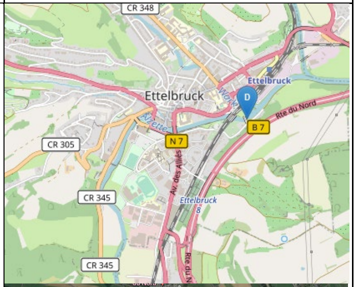
E68 **IT** **(Tos)**	**site:** MAS-VP4_TORRENTE STELLA few meters from site E49, but in river Stella Torrente. **catchment:** agriculture and urban **WWTP:** Quarrata (10-15,000 IE) discharge point into small creek connected to Stella Torrente			 
	United Kingdom			
E69 **UK**	**site:** TH-RSN1522, DS A34_River Cherwell at Kiddlington near Oxford, West Thames **catchment:** mixed agricultural and urban **WWTP:** Banbury (80,000 IE), Kings Sutton (no data)			 

	Luxembourg
	

72
71
Diekirch
Luxemburg
Lëtzebuerg
Luxembourg
Kanton Redingen
Canton Redange
Parc naturel de la Vallée de l'Attert
Kanton Mersch
Canton Mersch
Echternach
70
E25
E411
Arlon
Arel
A 7
Grevenmacher
A 1
Kanton Kapellen
Canton Capellen
Luxemburg
Luxembourg
73
Pétange
Petingen
Kanton Remich
Canton Remich
Remich
Longwy
Langich
Bettembourg
Bettemburg
Esch-sur-Alzette
Esch an der

<table>
<tr>
<td>E70</td>
<td>site: L202030A12 Syr at Mertert
catchment: 202 km²; agriculture dominating, smaller towns
WWTP: STEP_126_B001 Manternach (1,650 IE, tributary), STEP_122 _B001 Biwer (3,000 IE), STEP_122_B002 Betzdort-Habelsdorf (10,000 IE), STEP_406_B001 Uebersyren (35,000 IE)</td>
<td> L202030A12 Syr, Mertert</td>
<td> L202030A12 Syr, Mertert</td>
<td>  </td>
</tr>
<tr>
<td>E71</td>
<td>site: L100011A21 Alzette, Ettelbruck
catchment: 1,091 km²; urban/industrial headwater region (Esch-s.-A., city of Luxembourg), agriculture and forest dominating in lower catchment
WWTP: STEP_503_B001 Boevange/Attert (15,000 IE, tributary), STEP_509_B001 Mersch (70,000 IE), STEP_1_B001 Beggen (210,000 IE)</td>
<td> L100011A21 Alzette, Ettelbruck</td>
<td> L100011A21 Alzette, Ettelbruck</td>
<td>  </td>
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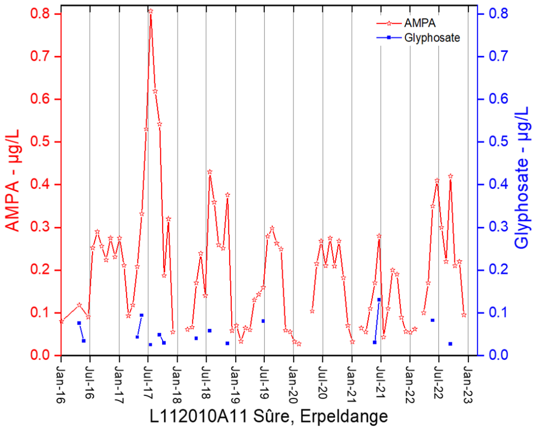
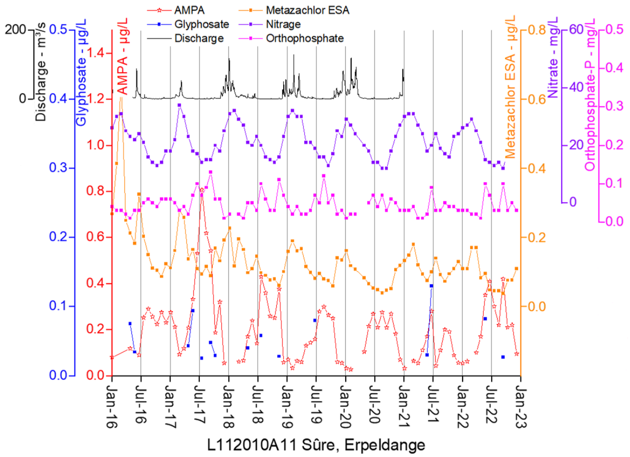
E72	**site:** L112010A11 Sûre, Erpeldange **catchment:** 947 km^2 (Michelau); agriculture and forests, relatively low population density **WWTP:** STEP_703_B002 Michelau (2,250 IE), STEP_703_B001 Bourscheid (2,500 IE, tributary), STEP_906_B004 Heiderscheidergrund (12,000 IE), several smaller WWTPs			

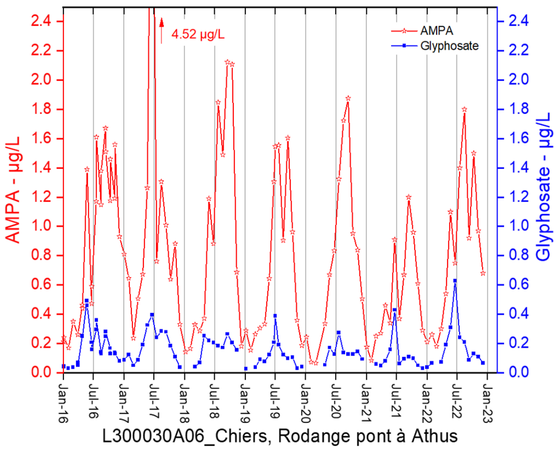
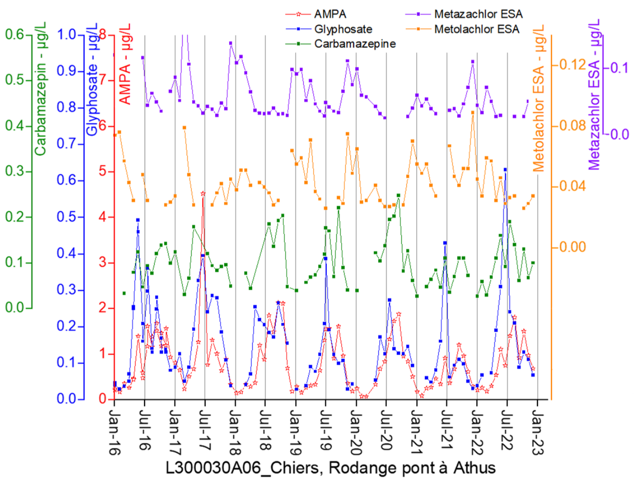
E73	**site:** L300030A06_ Chiers - Rodange pont à Athus **catchment:** 58 km² (Pétange); densely populated and industrialized, agriculture **WWTP:** STEP_309_ B001 Petange (70,000 IE)	 L300030A06_Chiers, Rodange pont à Athus	 L300030A06_Chiers, Rodange pont à Athus	

Table S1-3: Spearman rank correlation coefficients determined for glyphosate concentrations in correlation to concentrations of AMPA and of pesticides for different U.S. sites. Only pair-wise concentration data per sampling day were considered. Values <LOD were set to ½ LOD. Sites were those of Fig. 5, Fig. 6 and S1-1 and S1-3. The number of datapoints considered is given in brackets.

USA	**site number**		**herbicides**						**fungicide**
		AMPA	**meto-lachlor**	**dechloro-metola-chlor**	**atrazine**	**2,4-D**	**prometon**	**sulfen-trazone**	**azoxy-strobin**
Cherry Creek	U3	0.811 (229)				0.711 (167)	-0.194 (167)		
South Platte River	U4	0.039 (141)	0.632 (114)		0.140 (134)				
Red River	U5	0.872 (116)	0.649 (110)	0.456 (110)	0.640 (110)				
South Fork Iowa River	U7	0.728 (280)	0.667 (197)		0.062 (193)				
Maple Creek	U8	0.820 (267)	0.747 (176)	0.694 (175)	0.751 (172)				
Yazoo River	U10	0.491 (231)	0.337 (142)	0.128 (142)	0.071 (142)				
Sope Creek	U12	0.744 (188)						0.422 (148)	0.606 (150)

Table S1-4: Spearman rank correlation coefficients determined for glyphosate concentrations in correlation AMPA, chemicals derived from wastewater or pesticides for different European sites (sites used in Fig. 6, Fig. 7 and S1-1 and S1-2). Further details, see Table S1-3.

Europe	**site number**	**AMPA**	**carbama -zepine**	**analgesics**		**house-hold chem.**	**phos-phate**	**herbicides**			**fungi-cides**	**nitrate**
la Sambre Canalisée at Jeumont	E8	0.732 (108)				0.695 (60)[5]					0.585 (48)[15]	-0.504 (108)
l´Escaut Canalisé at Mortagne du Nord	E14	0.767 (129)		0.349 (72)[1]				0.206 (126)[9]	0.239 (90)[13]	0.066 (90)[13]		
Meuse at Bassoncourt	E15	0.157 (127)						0.102 (68)[8]	0.269 (71)[12]			
Seine at Conflans Sainte-Honorine	E18	0.705 (289)	0.204 (102)	0.296 (102)		0.416 (104)[6]		-0.194 (102)[8]	-0.345 (276)[10]			
Vistre de la Fontaine	E32	0.579 (43)		0.212 (25)[3]	0.487 (25)[2]		0.420 (43)	0.190 (66)[11]				
Sweden, N34	E39	0.381 (496)										
Neckar at Mannheim	E44	0.644 (231)	0.540 (207)				0.558 (220)	-0.411 (116)[7]				-0.126 (220)
Nahe at Bingen-Dietersheim	E47	0.679 (303)	0.593 (290)					-0.636 (196)[8]				
Main Bischofsheim, left shore	E56	0.852 (127)	0.552 (125)									
Main Bischofsheim, right shore	E56	0.639 (257)	0.362 (257)									
Syr at Mertert	E70	0.765 (77)	0.609 (69)				0.739 (77)	-0.103 (77)[8]				
Alzette at Ettelbruck	E71	0.689 (98)	0.678 (83)					-0.342 (90)[8]				
Chiers at Rodange pont à Athus	E73	0.765 (98)	0.637 (71)			0.152 (60)[5]	0.646 (81)	-0.640 (81)[7]				

[1] ibuprofen; [2] tramadol; [3] niflumic acid; [4] ethylenediaminetetraacetic acid (EDTA); [5] Tolyltriazole; [6] benzotriazole; [7] metolachlor sulfonic acid; [8] metazachlor sulfonic acid; [9] MCPA; [10] chlortoluron; [11] dimethenamid; [12] diflufenican; [13] chloridazon; [13] propyzamid; [14] propiconazol; [15] tebuconazol

Supplementary Information to Section 4

In-situ formation of glyphosate and AMPA in activated sludge from phosphonates used as antiscalants and bleach stabilizers in households and industry

S2-1 Synthesis of $^{13}C_5$-DTPMP

$^{13}C_5$-labeled DTPMP was synthesized according to a reaction scheme adapted from Winkler.[254] Briefly, in a 25 mL flask 1.476 g (18 mmol, 6 eq.) phosphorous acid were dissolved in 2.06 mL concentrated aqueous HCl (37%). The reaction mixture was cooled in an ice bath and 322.4 µL (3 mmol, 1 eq.) diethylenetriamine were added dropwise over a period of 1 hour. Subsequently, the reaction mixture was heated to 90 °C and 512 mg (16.5 mmol, 5.5 eq.) of ^{13}C-paraformaldehyde (99.9% ^{13}C) were added in small portions over a period of 2 h. After the addition of ^{13}C-paraformaldehyde, the temperature was increased to 110 °C and the reaction mixture was stirred for 4 h. The solvent was removed under reduced pressure using a rotary evaporator. The raw product was received as a yellow, highly viscous oil. The synthesis mixture was used after its characterization without further purification.

NMR data were recorded on a Bruker Avance III HD 400 spectrometer operating at 400.13 MHz for ^{1}H. ^{1}H and ^{31}P NMR measurements performed in D_2O (details in Section S2-2) revealed signals at: ^{31}P-NMR: [162.1 MHz, D_2O] δ(ppm): 12.89 (d, $^{1}J_{C\text{-}P}$ = 143.27 Hz, 1P), 9.43 (d, $^{1}J_{C\text{-}P}$ = 138.80 Hz, 4P) and ^{1}H-NMR: [400.13 MHz, D_2O] δ(ppm): 3.86-3.33 (m, 18H). The spectra are shown in Fig. S5.

$^{13}C_5$-labeled DTPMP and synthesis by-products with a lower degree of substitution (homologous mono, di, tri and tetra forms) were quantified using ion chromatography by CHT Germany GmbH, Tübingen, Germany, see Table S2-1. The mixture contained 40.5 wt% of $^{13}C_5$-DTPMP and significant percentages of the tri-form (20.7 wt%) and di-form (7.9 wt%). In total 71.3 wt% of the synthesized substance could be attributed to $^{13}C_5$-DTPMP and $^{13}C_5$-DTPMP-related amino(poly)phosphonates. Phosphite and chloride were present at a total concentration of 14 wt%. Table S2-1 also shows the ratio of the weight% of $^{13}C_5$-DTPMP and individual $^{13}C_5$-DTPMP-related amino(poly)phosphonates to their sum of 71.3 wt%. In addition, the molar masses of the different $^{13}C_x$-labeled substances are shown. These data are needed to calculate the average weighted molecular mass of all $^{13}C_x$-labeled amino(poly)phosphonates in the synthesis product (486 g/mol).

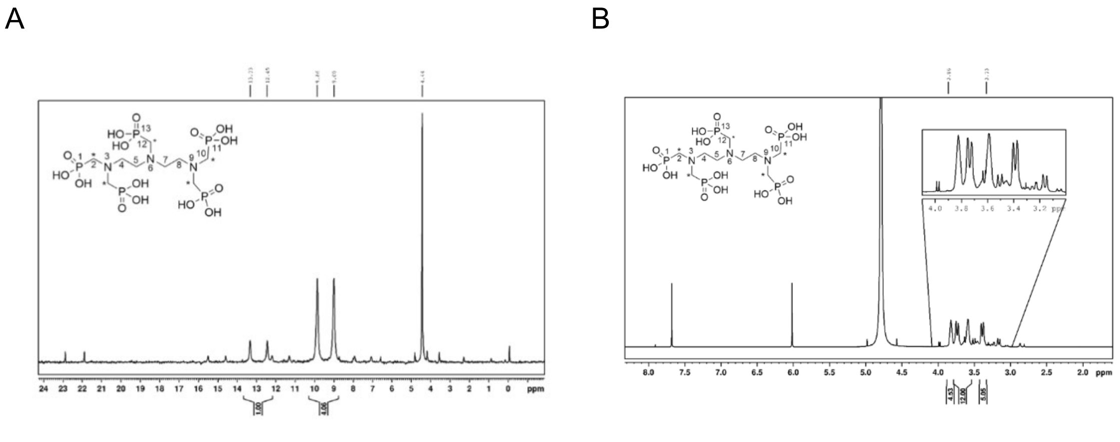


Fig. S5: (A) ^{31}P- and (B) ^{1}H-NMR spectra of $^{13}C_5$-DTPMP synthesized in-house and dissolved in D_2O, water signal at 4.79 ppm.

Table S2-1: Characterization of the mixture from the synthesis of $^{13}C_5$-DTPMP via ion chromatography by CHT Germany GmbH, Tübingen, Germany. The content in weight% in the synthesis mixture is provided. The third column shows the weight% of a specific isotope-labeled amino(poly)phosphonate relative to the total amount of isotope-labeled amino(poly)phosphonates with the ethylene diamine moiety in the mixture (in total 71.3 wt% in the mixture). All phosphonate carbons are ^{13}C-isotopes, which was considered when calculating the molar masses.

Analytes	**Content in weight%**	**Weight% of the total of amino(poly)phosphonates**	**Molar mass (g/mol)**
$^{13}C_5$-DTPMP	40.5	56.8	578
$^{13}C_4$-homologous – tetra form	1.6	2.2	483
$^{13}C_3$-homologous – tri-forms	20.7	29.0	388
$^{13}C_2$-homologous – di-forms	7.9	11.1	293
^{13}C-homologous – mono-forms	0.6	0.8	198
chloride	6.7	-	-
phosphite	7.5	-	-
phosphate	1.1	-	-
sum	86.6	100.00	486*

** as weighted average used for calculations*

DTPMP with all five phosphonate groups present, amounted to 40.5%. Further about 15 wt% were salts or byproducts (see Table S2-1). We added 1,475 mg/kg of the product mixture to activated sludge (experiments PW2-13C-DTPMP-0.6-BC, PW2-13C-DTPMP-0.6-AC). This equals a nominal starting concentration of about 596 mg/kg $^{13}C_5$-labeled DTPMP or 965 mg/kg total DTPMP and related amino(poly)phosphonates. For the latter, we considered all amino(poly)phosphonates, which could form glyphosate, thus considering all amino(poly)phosphonates with a central diethylenetriamine chain with one or more phosphonate groups. This total concentration of DTPMP-related products in the synthesis mixture of $^{13}C_5$-labeled DTPMP was 71.3 wt% and was used to assess formation rates of glyphosate and AMPA from DTPMP. For the total of $^{13}C_5$-labeled DTPMP and related amino(poly)phosphonates a weighted molar mass of 486 g/mol was calculated using Table S2-1. Considering that the dry weight of the activated sludge was 0.75% (factor of 133) of the sludge, we calculated the mol% (weight%) of labeled glyphosate and AMPA formed after 72 h and report the data as a range between mol% calculated for all ^{13}C-labeled amino(poly)phosphonates to considering only $^{13}C_5$-labeled DTPMP. We thus tried account for the uncertainty with expectable different reaction rates and different numbers of AMPA and glyphosate molecules theoretically available with regard to the chemical structure, see Figure 1C (for DTPMP: 2 glyphosate, 3 AMPA).

S2-2 Nuclear magnetic resonance spectroscopy

To determine the purity of the synthesis product $^{13}C_5$-DTPMP (for synthesis, see Section S2-1) ^{1}H-NMR- and ^{31}P-spectra were recorded by the NMR facility (Chemistry Department, University of Tübingen).

About 10 mg of the synthesis product $^{13}C_5$-DTPMP and 450 µL of deuterated water (D_2O) were mixed and vortexed for 5 s using an RS-VA 10 shaker (Phoenix Instrument, Garbse, Germany) and the mixture was transferred to an NMR glass tube. A Bruker Avance III HD 400 NMR spectrometer (Bruker cooperation, Billerica, MA, USA) was used, operating at

400.13 MHz for hydrogen observation with a zg30 pulse program and at 162.1 MHz for phosphorous observation with a zgpg30 pulse program. The acquisition parameters used for this experiment with 1D sequence with power-gated decoupling and a 30 ° flip angle were as follows for ^{1}H (^{31}P): number of scans: 64 (128), spectral width: 8,012.8 Hz (64102.6 Hz), offset (O1): 2,470.8 Hz (-8098.8 Hz), acquisition time: 4.09 s (0.51 s), relaxation delay (d1): 1.0 s (2.0 s). The spectra were evaluated quantitatively using the Bruker Top Spin 4.1.4 software.

S2-3 Analysis of centrifuged sludge samples

The amount of sample available from incubation experiments after freeze-drying was too low to be applicable for extraction. Thus, we pooled the samples from the incubation experiments run in triplicate. In order to demonstrate the differences between the triplicates, we also determined glyphosate and AMPA in centrifuged sludge. For this, aliquots of 2 mL were sampled at the time points indicated in Table 7 and centrifuged at 16,000 g for 10 min. The supernatant was discarded and the analytes glyphosate and AMPA were extracted from 200 mg (± 1%) of the pellet following the same protocol as described for freeze-dried samples with a liquid:solid ratio of 2.5. The weight of centrifuged samples was between 5.5-6.2% of the sludge used for incubation.

In general, results for centrifuged and freeze-dried samples were similar in the kinetics and relative concentrations of AMPA and glyphosate and are thus not discussed separately. The results are shown in Fig. S7 and Fig. S8.

S2-4 Additional figures and tables

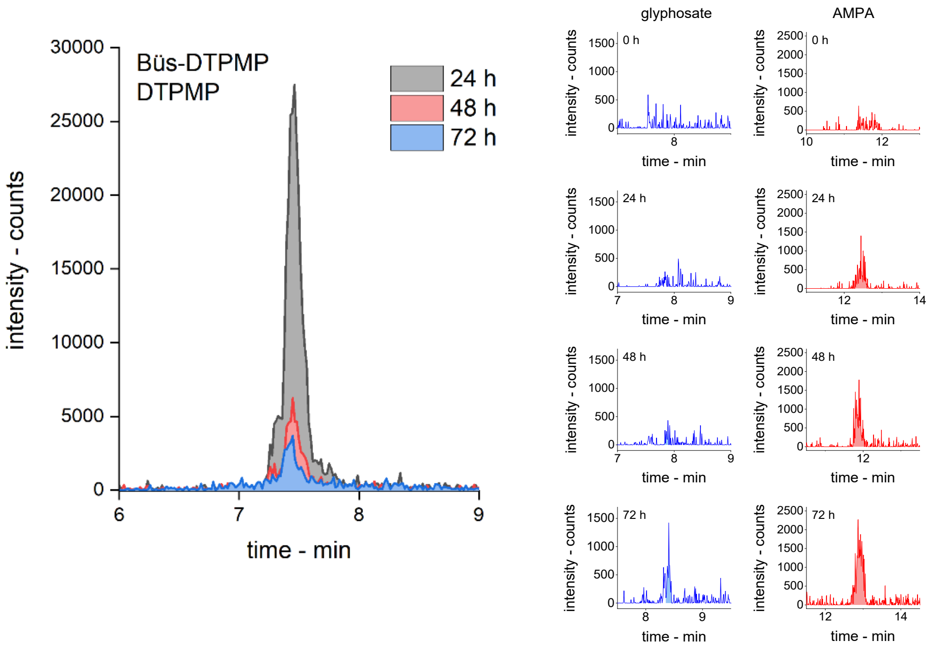


Fig. S6: Analytical results from DTPMP incubation in activated sludge sampled at the WWTP Büsnau; 600 mg/kg DTPMP incubated for 72 h (samples BÜS-DTPMP in Table 7). DTPMP analysis after extraction and methylation by LCMS-1; glyphosate and AMPA analysis by CE-MS without derivatization, see Section 4.3.4.2.

A

B

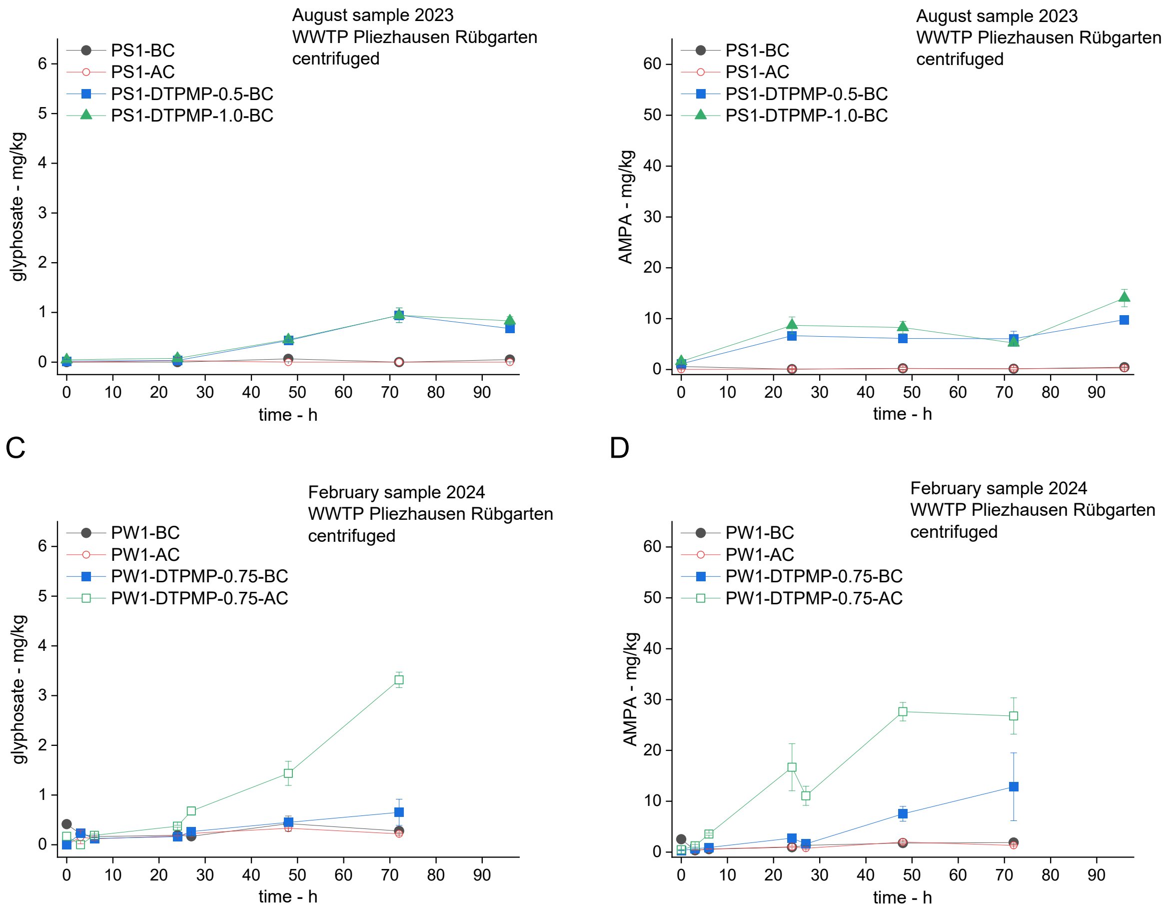


Fig. S7: Results of the same experiments as shown in Fig. 17A-D of the main text, but analyzing centrifuged instead of freeze-dried sludge samples (protocol in Section S2-3). All concentrations are given in mg/kg centrifuged sludge for triplicate experiments.

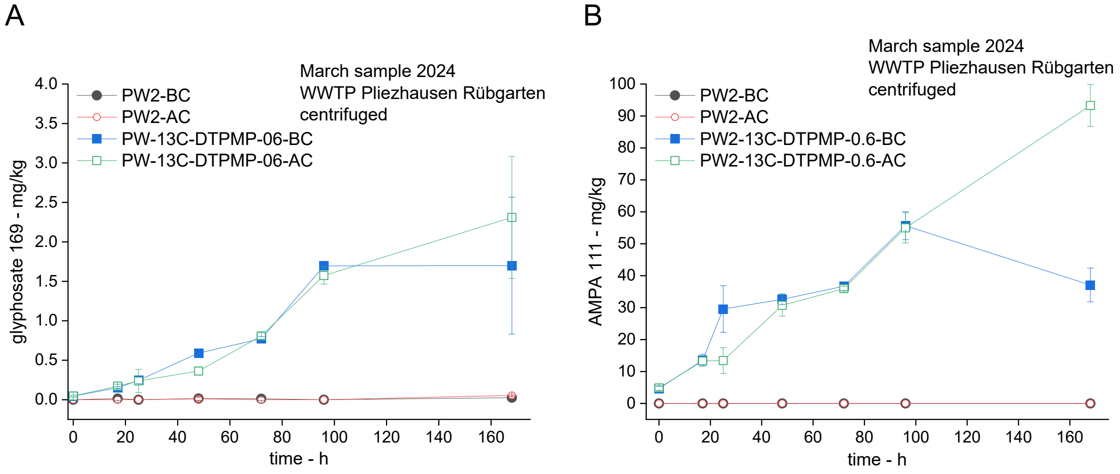


Fig. S8: Results of the same experiments as shown as in Fig. 19A and B of the main text, but analyzing centrifuged instead of freeze-dried sludge samples (protocol in Section S2-3). All concentrations are given in mg/kg centrifuged sludge for triplicate experiments.

Table S2-2: Glyphosate and AMPA formation from DTPMP in activated sludge (for experimental details, see Table 7). The formation is given as mol% in relation to the spiked concentration of DTPMP during incubation in fresh activated sludge using concentration data. All concentration values were corrected by the concentration determined in the controls at the same time point (measured after 72 h with CE-MS (see Table 9)). We determined 0.75% of the sludge to account for the dry weight, therefore DTPMP concentrations listed in Table 7 for the different experiments had to be multiplied by a factor of 133 to obtain the concentration in g/kg dry weight.

experiment		-BC		-AC	
		glyphosate formation in mol%	AMPA formation in mol%	glyphosate formation in mol%	AMPA formation in mol%
PW1-DTPMP-0.75		0.017	0.402	0.167	1.18
PS3-DTPMP-	0.05	0.019	1.35	0.024	1.33
	0.1	0.039	1.72	0.031	1.25
	0.25	0.040	1.21	0.032	1.39
	0.5	0.025	1.27	0.023	1.46