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Fungicides in English Rivers: Widening the Understanding of the Presence, Co-Occurrence and Implications for Risk Assessment

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Abstract: Fungicides are commonly found in freshwater; however, the understanding of their wider presence, co-occurrence, and potential risk remains limited. This study examined English national datasets to highlight knowledge gaps and identify improvements to monitoring and risk assessment. The analysis found that at least one fungicide was present in 91% of samples collected from English rivers over a 5-year period, with four fungicides detected at rates exceeding 50%. Co-occurrence occurs widely, with up to nine different fungicides detected within the same sample and four detected the most frequently, raising concerns for synergistic interactions. The semi-quantitative nature of much of the available data precludes a clear determination of the potential risk of detrimental effects on aquatic biota. Fully quantitative analysis is required, and ecotoxicity-based water quality standards need to be agreed upon. The monthly sampling regime reflected in the national datasets will infrequently capture high flow events and so is unlikely to fully represent fungicides transported to rivers via rainfall-driven processes. Several information gaps exist, including the risk posed by fungicides in sewage sludge applied to land and the extent to which fungicides in the aquatic and terrestrial environments contribute to antifungal resistance. Improvements in spatial and temporal information on fungicide use are needed.

Keywords: fungicides; freshwater; mixtures; monitoring; risk assessment

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1. Introduction

1.1. Sources and Pathways

Fungicides are used in agriculture, pharmaceuticals, personal care products, and for material preservation. Their application in agriculture is to control disease and safeguard the yield and quality of crops [1] including grapevines [2], while they also have amenity use being applied, for example, to golf courses and gardens [3,4]. Fungicides are used as antifungal active ingredients within human pharmaceuticals, often administered as topical or oral medicines and within personal care products, including shampoos, soaps, toothpastes, and shower gels [5]. Fungicide use for material preservation includes paints and coatings on facades and treated wood, walls and wallboards, and flat roofs and basement seals [6–8]. Fungicides enter aquatic ecosystems from all these uses, including indirectly via effluent from wastewater treatment plants [9] and runoff directly connected to the sewer system. Non-point agricultural pathways, including via spraying, leaching and runoff [10], and urban stormwater [11] are also key and are rainfall-driven processes [12].

1.2. Detection in Aquatic Systems

While fungicide occurrence, fate, and effects have historically received less focus than insecticides and herbicides, a growing number of studies have shown them to be detected in both agricultural and urban surface waters [10]. Among the presence of numerous organic pollutants in two chalk streams draining predominantly agricultural land in Southern England, 25 fungicide compounds were detected [13]. This equated to 32% of the 88 plant protection products identified. Detection rates were highest in March, reflecting springtime application to crops. These findings were in broad agreement with a study that used passive samplers to characterize plant protection products in a river in southeast England [14], where fungicides accounted for 26.5% of the total 128 pesticides detected. In a catchment study in southwest England, effluent from wastewater treatment plants was found to be the key pathway for human and household fungicides entering the riverine environment, with diffuse source agricultural fungicides also detected [15]; an environmental assessment using a risk quotient approach determined that several of the fungicides detected posed a medium or high risk to aquatic life. In Germany, a comprehensive study of pesticides in small streams draining agricultural land detected a total of 109 fungicides [16] and found that the average number of exceedances of regulatory acceptable concentrations grew as the area of agricultural land drained increased.

While the presence of fungicides in freshwater is now receiving greater attention, studies are typically limited to the river or catchment scale and are therefore influenced by local fungicide use. Moreover, these studies have not generally addressed co-occurrence or temporal variation, with only a limited number quantifying risk.

1.3. Effects on Freshwater Biota

In aquatic systems, fungicides can be toxic to a wide range of non-target organisms as they act on basic biological processes that are not specific to fungi [17]. A comprehensive review [10] highlighted the widespread occurrence of fungicides in aquatic systems at concentrations that can cause adverse, toxic effects on several groups of organisms. Significant sublethal effects of fungicides are reported, including effects on fish reproduction [18], immune response, zooplankton community composition, metabolic enzymes, and ecosystem processes, such as leaf decomposition in streams [19]. In zebrafish, exposure to fungicides causes reduced locomotion, which can be either direct toxicity or a sedative effect [20], and has the potential to negatively impact survival and population growth by impeding foraging, reducing reproduction, and increasing susceptibility to predation. Toxic effects on algae, daphnia, and fish have arisen from strobilurin fungicides used in agriculture, including mitochondrial and immunotoxicity, with the potential for endocrine disruption [21]. Regulatory risk assessments for fungicides in freshwater ecosystems do not specifically address toxicity towards freshwater fungi [22]. This would appear to be an important omission given the importance of fungi to key ecological functions. Chytrids, for example, are pathogenic fungi that infect cyanobacteria, the organisms that form toxic algal blooms. Environmentally relevant concentrations of the fungicides azoxystrobin and tebuconazole have been shown to cause significant reductions in chytrid infection of cyanobacteria, elevating the risk of bloom formation [23].

1.4. Mixture Toxicity and Synergism

Mixtures of azole fungicides have been shown to have both synergistic and additive toxic antiandrogenic effects, highlighting a need to account for this in hazard and risk assessments [24]. Recommendations for the risk assessment of azole fungicides have suggested cumulative approaches due to their shared mode of action and propensity for mixture toxicity [25,26]. Fungicides have also been identified as contributing to synergistic or additive interactions within mixtures of other chemical types [27], leading to detrimental impacts upon aquatic life [28,29]. Within reported synergistic mixtures of pesticides, azole fungicides have been reported as the second most commonly implicated pesticide class [25].

1.5. Human Health and Antifungal Resistance

An environmental transformation product of several azole fungicides has been detected in Danish groundwater that provides a raw drinking water source [30], while fungicides are reported in source, treated, and tap water in Central China [31]. In France, a degradation product of the fungicide chlorothalonil has been widely detected in raw drinking water despite it being banned there in 2020 [32]. The presence of fungicides in drinking water raises implications for human health, particularly given their potential for endocrine disruption and growing concerns regarding antifungal resistance [33].

Azole fungicide-resistant strains of Aspergillus fumigatus are thought to have emerged due to agricultural fungicides [34]. Such strains have been linked to cases of resistant fungal infections in patients with no previous medical azole fungicide exposure, suggesting the environment as a source of resistant strains. Worryingly, medical azole fungicides, key in treating such infections, share the same modes of action with those used as pesticides, so emerging environmental resistance presents global human health threats [34,35]. As such, there is growing interest in tools for estimating threshold concentrations for the emergence of resistance [36], but there is currently no agreed upon method. In any case, these risk assessments and monitoring tools need to be underpinned by robust monitoring schemes to allow the risk to be properly characterized.

1.6. Purpose of the Paper

Building on recent studies focused on specific rivers, one purpose of this study was to undertake a first national analysis of fungicides in English rivers, examining their frequency of detection, temporal variation, and co-occurrence both with other fungicides and with known synergists. Additionally, where possible, assessment against ecotoxicological standards is made, with relevance to the implementation of the European Commission's Water Framework Directive [37]. The challenge of assessing the risk for pollutants for which agreed upon standards are not established is also addressed. A second key purpose was to identify knowledge gaps and propose improvements to monitoring and risk assessment of fungicides in aquatic systems. The monitoring recommendations arising are likely to be of wider relevance, given that the UK ranked 35th in global pesticide use in 2021 [38] and that analysis of the monitoring of aquatic environments across the European Union found a lack of fungicide data [26].

2. Methods—Data Sources and Analysis

Four separate sources of data were used to undertake the analysis described in this paper. The fungicides associated with each source are summarized in the Supplementary Information (Table S1).

2.1. LC-MS and GC-MS Databases

The Environment Agency, the public body with responsibilities relating to the protection and enhancement of the environment in England, has been using Gas Chromatography–Mass Spectrometry (GC-MS) and Accurate-mass Quadrupole Time-of-Flight (Q-TOF)/Liquid Chromatography–Mass Spectrometry (LC-MS) target screening analysis [39] to measure organic substances in groundwater and surface water since 2009 for GC-MS and since 2014 for LC-MS. A single calibration is used for each analyte in the LC-MS and GC-MS analyses, and this, coupled with other uncertainties, means the data are semi-quantitative and do not reflect a fully validated concentration. The limit of detection (LoD) for all LC-MS analyses was 0.001 µg/L, and for GC-MS, it was 0.01 µg/L for all compounds, except for fluoxastrobin, which was 1 µg/L. Sampling for LC-MS and GC-MS analysis is undertaken at a less than monthly frequency and, as such, is likely to introduce a bias to fungicide concentrations found at low to mean flow.

The Environment Agency's Prioritisation and Early Warning System (PEWS) provides a set of prioritization scores for chemicals of emerging concern based on a sifting and screening process [40]. The 2023 PEWS assessment provides a prioritization for 19 fungicides (Table 1), with all but one of these being used in agriculture, the exception being triclosan, which is used as an antimicrobial (effective against both bacteria and fungi) in over-the-counter cosmetic products, personal hygiene products, and food packaging. Of the 19 fungicides, 2 are now banned, epoxiconazole and propiconazole, while a withdrawal from the market is proposed for mancozeb [41].

Table 1. Overview of the 19 PEWS-identified fungicides used in the LC-MS and GC-MS analyses. PEWS Surface Water Risk: Priority 1—high risk, high certainty; Priority 2—high risk, low certainty; Priority 3—low risk, low certainty; Priority 4—low risk, high certainty. Approval status is provided by the Biocidal Products Regulation for Great Britain and Northern Ireland [41].

Chemical Name	Approval Status	Fungicide Class	PEWS Surface Water Risk
Azoxystrobin	Approved	Strobilurin	Priority 4
Captan	Approved	Dicarboximide	Priority 2
Dimoxystrobin	Approved	Strobilurin	Priority 1
Epoxiconazole	No longer approved	Azole	Priority 1
Fludioxonil	Approved	Phenylpyrrole	Priority 2
Fluoxastrobin	Approved	Strobilurin	Priority 2
Fluquinconazole	Approved	Azole	Priority 2
Imazalil	Approved	Azole	Priority 4
Ipconazole	Approved	Azole	Priority 4
Mancozeb	Withdrawal due 2025	Carbamate	Priority 2
Metalaxyl-M	Approved	Acylalanine	Priority 4
Metconazole	Approved	Azole	Priority 1
Penconazole	Approved	Azole	Priority 1
Penthiopyrad	Approved	Pyrazole carboxamide	Priority 2
Prochloraz	Approved	Azole	Priority 4
Propiconazole	No longer approved	Azole	Priority 1
Proquinazid	Approved	Quinazolinone	Priority 4
Tebuconazole	Approved	Azole	Priority 1
Triclosan	Banned in some products	Chlorinated aromatic	Priority 1

The 19 fungicides were each searched for in the LC-MS and GC-MS databases for the years 2019 to 2023 inclusive (Table 1) for English rivers, to provide an overview of the detection rates and co-occurrence within the same sample and monitoring site. Furthermore, a review of the literature was undertaken to determine fungicide combinations known to exhibit synergistic toxic effects, and the occurrences of these combinations were then searched for in the same sample and at the same site within each year in the LC-MS database. Both the LC-MS and GC-MS datasets provide data for the whole year for 2019 to

2022, inclusively, but, at the time of data collation, data for 2023 were only available up to June of that year.

2.2. Water Quality Archive (WQA)

The Environment Agency's Water Quality Archive [42] contains water quality measurements across England that are validated, fully quantitative concentrations. Some of the data are used to compare against standards to determine waterbody classification status under the European Commission's Water Framework Directive [37]. Four fungicides, triclosan, azoxystrobin, metaxalyl, and prochloraz, are named under the WQA. Triclosan has been added to the proposed updated list of priority substances [43,44] and, as such, has associated proposed environmental quality standards (EQSs), i.e., an annual average (AA) and maximum allowable concentration (MAC) in the water column to protect the aquatic environment. Both the proposed AA and MAC for triclosan are 0.02 µg/L for inland surface waters. Azoxystrobin is neither an existing nor proposed priority substance but is proposed as a candidate substance under the European Commission's fourth watch list, with an associated proposed Predicted No Effect Concentration (PNEC) of 0.2 µg/L [45]. The watch list was established under the Water Framework Directive with the aim of improving the available information on identifying the substances of greatest concern. Neither metaxalyl nor prochloraz is classified as a priority substance or a proposed priority substance. Both triclosan and azoxystrobin were searched for in the WQA database for the years 2019 to 2023 inclusive for English rivers, and the results were compared against their respective designated EQSs and PNECs. To determine the AA EQS for triclosan, samples that were less than the LoD were set to half the LoD and included within the calculation of the annual average at that site. WQA LoDs are provided via [42]. As with the monitoring for the LC-MS and GC-MS analyses, sampling for the WQA was undertaken at typically a less than monthly frequency.

2.3. UKWIR Chemical Investigations Programme

The UK Water Industry Research's (UKWIR) Chemical Investigations Programme (CIP) provides data on a range of chemicals in sewage effluent and sewage sludge (biosolids) [46]. From the UKWIR dataset [47], the concentrations of fungicides in treated effluent and sewage sludge were extracted. Data from 2019–2022 were extracted, as 2023 data were not available at the time of download.

2.4. Data Processing and Statistics

All data extraction, manipulation, and statistical analysis was performed using R version 4.4.0 [48]. Data normality was checked in R. The Wilcoxon rank sum test was selected for its statistical power when working with skewed distributions. Rather than using original values, this test ranks data points, allowing for statistically significant differences in median values to be determined between population distributions. Tests were performed in R to compare fungicide concentrations between data sources.

3. Results

3.1. LC-MS and GC-MS

Detection frequencies and concentrations reported in the LC-MS dataset are provided in Table 2. The number of samples collected for LC-MS analysis varied between years (423 in 2019, 116 in 2020, 362 in 2021, 549 in 2022, and 146 in 2023 up to and including June). The median number of samples taken per site per year ranged from 1 to 10. Of the 1596 samples taken over the 2019–2023 period, there were 1446 detections (91%) of at least one fungicide. Seven fungicides (azoxystrobin, epoxiconazole, fludioxonil, fluoxastrobin, propiconazole, tebuconazole, and triclosan) were detected at rates of between 27% and 82% with respect to the total number of samples taken for LC-MS analysis. The remainder were either detected at no greater than 2% or, in the case of fluquinconazole, ipconazole, and proquinazid, were not detected at all. Neither captan nor mancozeb was assessed under LC-MS. The reported concentrations are strongly caveated by the semi-quantitative nature of the data; however, the highest single measured concentration of any fungicide was propiconazole at 9 μg L^{-1}, and the highest mean concentration was for triclosan at 0.672 μg L^{-1}.

Table 2. Number of LC-MS detections, detection frequencies, and summary of concentrations for PEWS fungicides from January 2019 to June 2023 inclusive. [a] = Total samples taken (1596), [b] = Total number of samples where fungicides were detected (1446). Fluquinconazole, ipconazole, and proquinazid were not detected. Neither captan nor mancozeb was assessed under LC-MS. * For metalaxyl, the LC-MS database does not report results for specific enantiomers, so detections may reflect both R- and S-enantiomers.

Compound	Detections	Detection [a] (%)	Detection [b] (%)	Concentration (μg L^{-1})			
				Median	Mean	Min	Max
Azoxystrobin	1303	82	90	0.0061	0.0208	0.0011	2.2
Dimoxystrobin	7	0.4	0.5	0.013	0.0126	0.0037	0.02
Epoxiconazole	730	46	50	0.0029	0.00481	0.0011	0.05
Fludioxonil	430	27	30	0.0016	0.00393	0.0011	0.22
Fluoxastrobin	516	32	36	0.002	0.00287	0.0011	0.026
Fluquinconazole	0	0	0	-	-	-	-
Imazalil	30	1.9	2.1	0.00135	0.00167	0.0011	0.0034
Ipconazole	0	0	0	-	-	-	-
Metconazole	4	0.3	0.3	0.0031	0.00615	0.0014	0.017
Metalaxyl *	10	0.6	0.7	0.00825	0.0152	0.0012	0.045
Penconazole	2	0.1	0.1	0.00145	0.00145	0.0014	0.0015
Penthiopyrad	21	1.3	1.5	0.0015	0.00168	0.0011	0.0035
Prochloraz	9	0.6	0.6	0.0032	0.00904	0.0011	0.049
Propiconazole	807	51	56	0.0029	0.0155	0.0011	9
Proquinazid	0	0	0	-	-	-	-
Tebuconazole	813	51	56	0.0082	0.0183	0.0011	1.1
Triclosan	857	54	59	0.0052	0.0672	0.0011	5

Substantially more samples were collected for GC-MS analysis (6460) compared to LC-MS (1596) over the 2019–2023 period; however, fewer fungicides were detected and with lower detection frequencies (Table 3). Additionally, when detected, the concentrations under GC-MS are typically higher than those under LC-MS. These findings may relate to the poor detection of some polar compounds by GC-MS [49] and the relatively high GC-MS detection limits [50]. The most frequently GC-MS-detected fungicide was tebuconazole, found in 1.56% of all samples collected and 56% of samples where any one of the 19 PEWS fungicides was detected. The highest concentration in this dataset was seen for the detection of fluoxastrobin at 9.8 μg L^{-1}. Other than single detections for captan and fluoxastrobin, the highest mean concentration was found for azoxystrobin at 0.187 μg L^{-1}. There were no detections for imazalil, ipconazole, penconazole, penthiopyrad, and prochloraz. Again, the interpretation of concentrations is to be treated with caution given the semi-quantitative nature of the data. Mancozeb and metconazole were not assessed under GC-MS.

Table 3. Number of GC-MS detections, detection frequencies, and summary of concentrations for PEWS fungicides from January 2019 to June 2023 inclusive. [a] = Total samples taken (6460), [b] = Total number of samples where fungicides were detected (181). Mancozeb and metconazole were not assessed under GC-MS.

Compound	Detections	Detection [a] (%)	Detection [b] (%)	Concentration (μg L^{-1})			
				Median	Mean	Min	Max
Azoxystrobin	31	0.48	17	0.052	0.187	0.019	1.7
Captan	1	0.02	0.6	0.47	0.47	0.47	0.47
Dimoxystrobin	4	0.06	2.2	0.046	0.063	0.029	0.131
Epoxiconazole	17	0.26	9.4	0.043	0.051	0.018	0.163
Fludioxonil	21	0.33	12	0.03	0.048	0.011	0.208
Fluoxastrobin	1	0.02	0.6	9.8	9.8	9.8	9.8
Fluquinconazole	2	0.03	1.1	0.0645	0.065	0.048	0.081
Imazalil	0	0	0	-	-	-	-
Ipconazole	0	0	0	-	-	-	-
Metalaxyl	27	0.4	13	0.039	0.129	0.007	0.8
Propiconazole	32	0.50	18	0.0805	0.148	0.013	0.995
Penconazole	0	0	0	-	-	-	-
Penthiopyrad	0	0	0	-	-	-	-
Prochloraz	0	0	0	-	-	-	-
Tebuconazole	101	1.56	56	0.02	0.136	0.011	9
Triclosan	18	0.28	9.9	0.0155	0.022	0.011	0.066

3.2. Co-Occurrence of Fungicides by Site and Sample

To determine co-occurrence in the LC-MS database, the total number of unique fungicides detected per sample and at each sampling site across a given year were both quantified. The highest number of unique fungicides detected in a single given sample was nine, with four being the most frequently detected (Figure 1A). The highest number of fungicides detected annually at a given site was nine, although the variation is marked, with one, six, and three fungicides being the three most commonly observed co-occurrences (Figures 1B and 2).

In addition to mixtures of fungicides themselves, their co-occurrence with other chemicals has also been shown to give rise to mixture effects. For example, propiconazole potentiates the effects of clothianidin and dimethoate insecticides in bees [51], while the neonicotinoid insecticide imidacloprid was found to have additive and synergistic effects towards multiple invertebrate taxa when combined with the azole fungicide prochloraz [52]. Searching the LC-MS database (riverine data) for chemical combinations in the same sample known to exhibit synergistic toxic effects (Table 4) revealed rates of detection of propiconazole in combination with imidacloprid (42% of total samples) and clothianidin (32% of total samples), and the combination of tebuconazole and clothianidin was 43%.

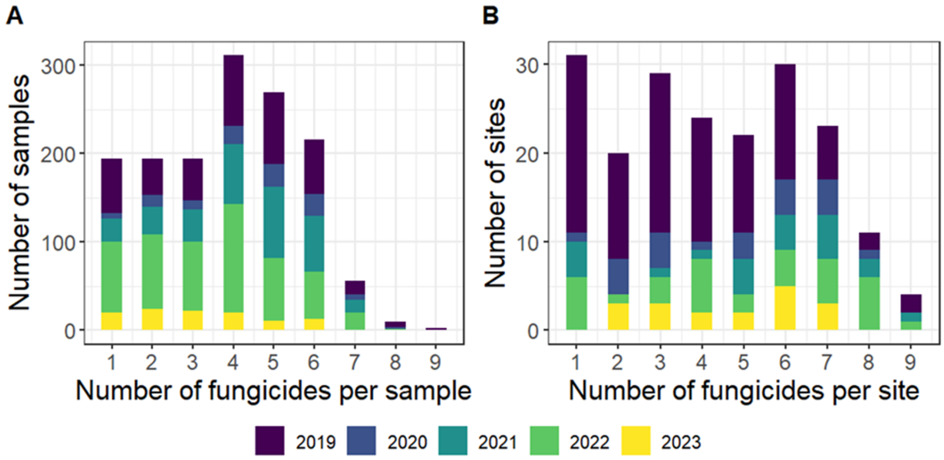

Figure 1. (**A**) Histogram showing number of unique fungicides found in a given sample between 2019 and 2023. (**B**) Histogram showing number of unique fungicides detected each year at a sampling site.

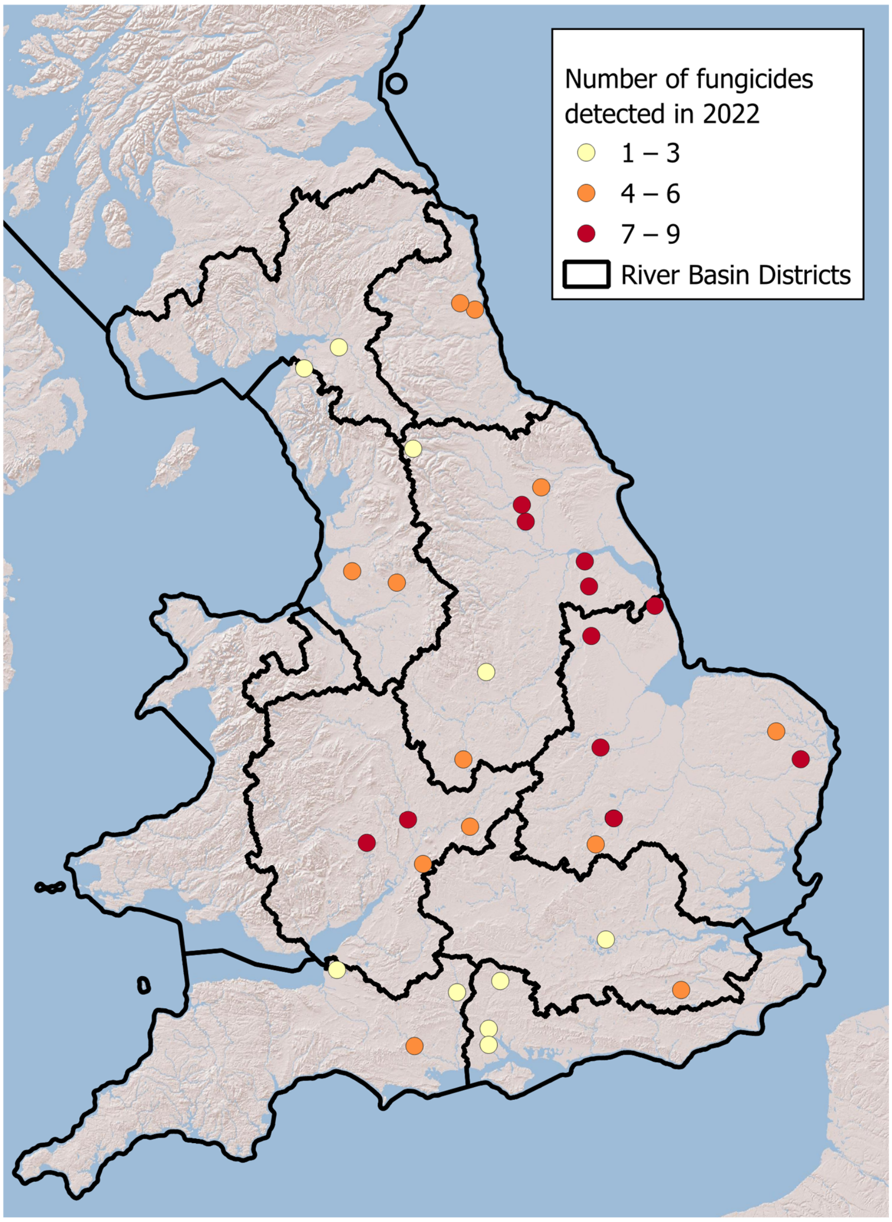

Figure 2. Location of fungicide detection, including co-occurrence, under LC-MS during 2022. Data are derived from the Environment Agency's LC-MS database.

Table 4. Same sample co-occurrence frequency of fungicides with insecticides known to exhibit synergistic toxic effects in combination. * Given the low detection frequency of prochloraz in the LC-MS dataset and the shared mode of action of azole fungicides, the co-occurrence with propiconazole was also assessed to further identify potential synergistic effects, although this specific combination is not alluded to by [52].

	Number of Samples	% Total Samples	Reference
Prochloraz + Imidacloprid	4	0.3	[52]
Propiconazole + Imidacloprid *	673	42	
Propiconazole + Clothianidin	523	32	[51]
Propiconazole + Thiamethoxam	82	5	[53]
Tebuconazole + Clothianidin	681	43	[53]
Tebuconazole + Thiamethoxam	133	8	[53]

3.3. Environmental Persistence of a Banned Fungicide—Epoxiconazole

Authorizations for the use, storage, and disposal of epoxiconazole ended in 2021, and to explore the impact of the ban on its presence in rivers, annual statistics were compared across all LC-MS sampling points (Figure 3). No significant difference by the Wilcoxon rank sum test was found between 2019 and 2020 before the year of the ban. In 2021, however, when the ban was introduced and during the subsequent years, there are significantly lower concentrations of epoxiconazole observed compared to both 2019 and 2020. However, no significant difference was found between the years of 2021, 2022, and 2023, indicating a plateau in the reduction of epoxiconazole concentration in rivers and an apparent persistence in the environment following the ban, although this is caveated by the semi-quantitative nature of the data, the variability in the number of samples taken each year due to the COVID pandemic, and incomplete data availability for 2023. The solubility of epoxiconazole in water is relatively low, and its persistence in soil is high [54]. Continued and consistent detections following the ban may, therefore, indicate a gradual leaching from contaminated soils into surface waters.

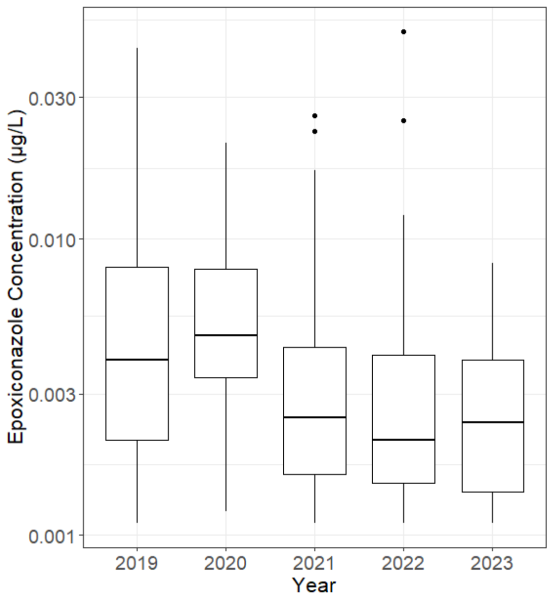

Figure 3. Boxplots of detected epoxiconazole concentrations by LC-MS for 2019 (n = 227), 2020 (n = 85), 2021 (n = 212), 2022 (n = 163), and 2023 (n = 43). The boxes represent the upper and lower quartiles, the horizontal bars represent the median, the whiskers represent the minimum and maximum (excluding outliers), and the dots represent outliers.

3.4. Monthly Variability

The discrimination of seasonal patterns in the fungicide presence and magnitude of the concentration can help to inform the design of monitoring programs, indicating the efficacy of current monitoring schemes and whether, for example, greater resources may need to be targeted at particular compounds at certain times of the year to capture peaks during windows of use. To explore this, the mean LC-MS riverine concentrations across all sites of four fungicides were determined on a monthly basis for the years 2019, 2021, and 2022, which were selected because they had the most complete datasets with the highest detection frequencies for multiple fungicides. Mean monthly concentrations for the four, namely, azoxystrobin, epoxiconazole, fludioxonil, and fluoxastrobin, are illustrated in Figure 4. While these results are caveated by the semi-quantitative nature of the data, it is the relative variation in detection and concentration that is of key interest here rather than the absolute concentrations.

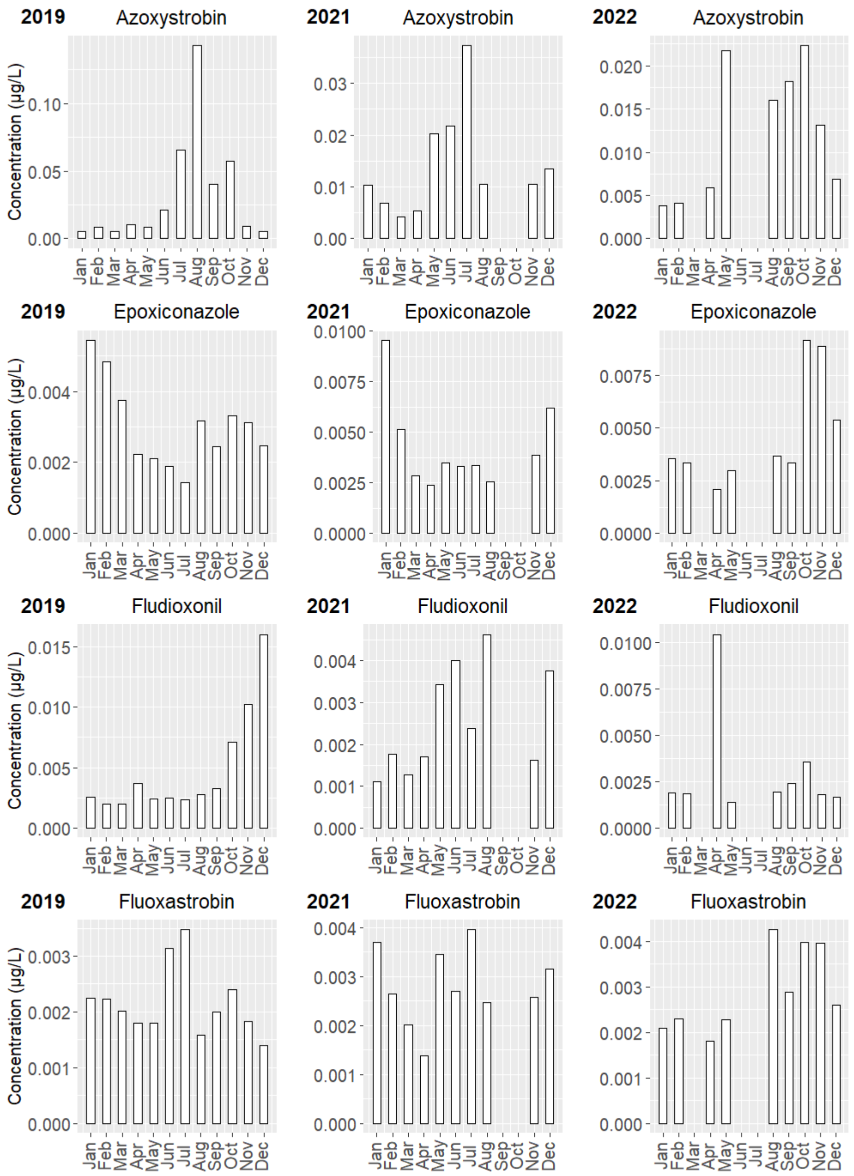

Figure 4. Bar plots showing the monthly average LC-MS concentrations of selected fungicides for 2019 (*n* = 349 for azoxystrobin, 227 for epoxiconazole, 116 for fludioxonil, and 157 for fluoxastrobin), 2021 (*n* = 297 for azoxystrobin, 212 for epoxiconazole, 65 for fludioxonil, and 153 for fluoxastrobin), and 2022 (*n* = 470 for azoxystrobin, 163 for epoxiconazole, 205 for fludioxonil, and 109 for fluoxastrobin). Absence of bars indicate no detections. Data used were >LoD only.

Azoxystrobin showed marked peaks in the summer months during 2019 and 2021 and both late spring and autumn in 2022, indicating that riverine concentrations are likely to be primarily driven by its use during summer crop growth. For the banned fungicide epoxiconazole, peaks occurred in the winter months when rainfall is generally highest, supporting the theory that runoff of legacy stores, built up within the soil over previous years of use, continues to occur. Fludioxonil showed no well-defined pattern across the 3 years. This may be due to its varied use, including post planting of winter and summer crops, for the preservation of seed stocks, and application to fruit and vegetables post-harvest both during transport and at the point of sale [55]. Its detection in the aquatic environment therefore reflects both discharge via wastewater treatment plants and runoff from agricultural land. Fluoxastrobin showed a relatively consistent concentration throughout the year, with less defined peaks. This probably reflects its versatile use as a seed treatment and a preventive treatment for different fungal infections in a range of crops, including winter crops [54]. The variation in fungicide use between years that is reflected within these data will be strongly influenced by climatic variability, particularly moisture, heat, and humidity, that affect the emergence and virulence of pests and therefore the demand for chemical control [56]. These factors do not affect crops uniformly, and hence, subsequent fungicide use will vary spatially and temporally. Additionally, river flows will impact the degree of dilution and, hence, the observed riverine fungicide concentrations.

3.5. WQA—Evaluation of Fully Quantitative Data Against Standards

Statistics describing the presence and exceedance against their respective EQSs and PNEC standards are provided for triclosan and azoxystrobin in Tables 5 and 6, respectively. The median number of samples collected per site per year varied between 1 and 10, and 1 and 11 for the analysis of triclosan and azoxystrobin, respectively. In both cases, therefore, a monthly spot sampling regime, at best, has been adopted. Detection of triclosan above the limit of detection reaches no higher than 13% (in 2019) with tentative evidence of a decrease in presence since then. Similarly, the exceedance of the annual average EQS reached a maximum of five in 2019, reaching no more than one per year since. The exceedance of the maximum allowable concentration EQS is more variable, reaching a maximum of 21 in 2019 with 10 occurrences in 2022. Sampling for azoxystrobin increased sharply in 2021, with the number of samples exceeding the limit of detection following a similar trend. The exceedance of the PNEC is rare, with two samples representing the maximum annual occurrences observed. Data for metaxalyl and prochloraz were all below the limit of detection.

Table 5. Summary WQA statistics for triclosan.

Triclosan	2019	2020	2021	2022	2023
Total sites sampled	96	92	97	131	132
Total number of samples	587	134	878	1118	308
Min samples per site	1	1	1	1	1
Max samples per site	7	3	12	12	4
Median samples per site	7	1	10	10	3
Samples > Limit of Detection	76	3	34	83	4
% Samples > Limit of Detection	13	2	4	7	1
AA EQS exceedances	5	1	0	1	0
% of AA EQS exceedances	5	1	0	1	0
MAC EQS exceedances	21	1	3	10	0
% of MAC EQS exceedances	4	1	<1	1	0

Table 6. Summary WQA statistics for azoxystrobin.

Azoxystrobin	2019	2020	2021	2022	2023
Total sites sampled	4	3	101	101	208
Total number of samples	23	8	885	1001	1417
Min samples per site	3	2	1	1	1
Max samples per site	7	3	12	13	12
Median samples per site	6.5	3	9	11	7
Samples > Limit of Detection	0	0	14	8	50
% Samples > Limit of Detection	0	0	2	1	4
PNEC exceedances	0	0	1	1	2
% of PNEC exceedances	0	0	<1	<1	<1

3.6. Comparison Between LC-MS and WQA Data

Both azoxystrobin and triclosan are present in both the LC-MS and WQA datasets, enabling a comparison between them to be made. With the exception of triclosan in 2020, Wilcoxon rank sum tests revealed significantly higher concentrations in the WQA than the LC-MS dataset for both azoxystrobin ($p < 0.0001$) and triclosan ($p < 0.0001$–0.01) for samples where these compounds were present at detectable concentrations (Figure 5). These results suggest that, given the semi-quantitative nature of the LC-MS data, they may underestimate the concentrations of these two fungicides.

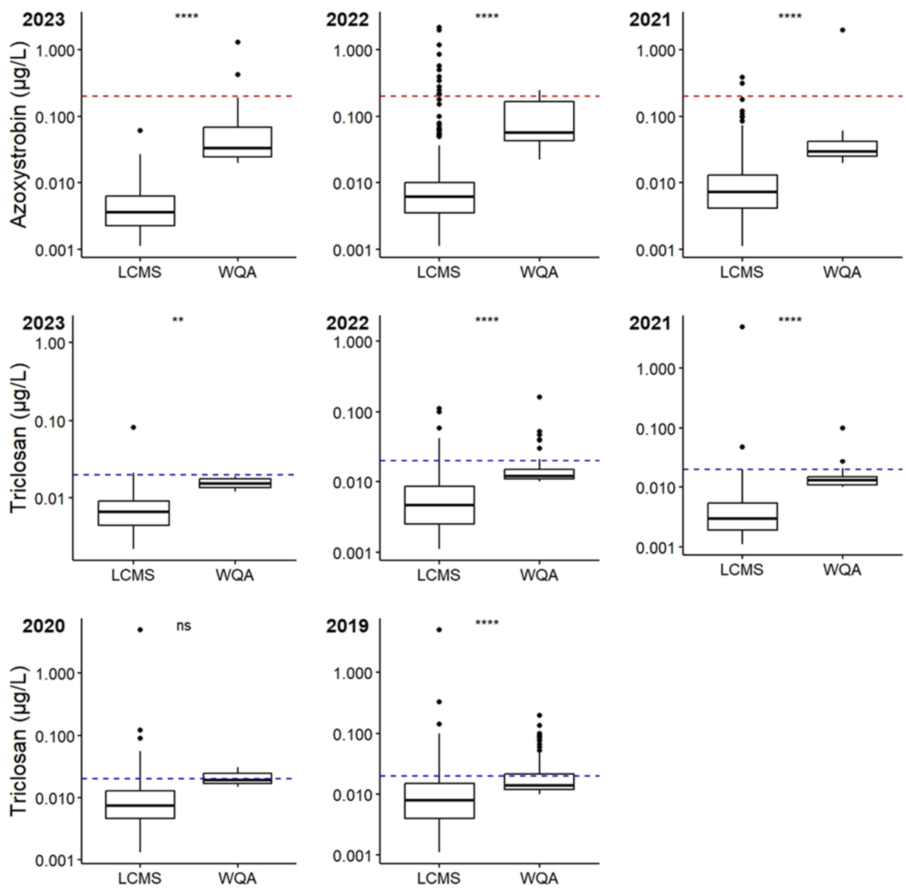

Figure 5. Boxplots of concentrations of azoxystrobin and triclosan from the LC-MS and Water Quality Archive (WQA) datasets. The boxes represent the upper and lower quartiles, the horizontal bars represent the median, the whiskers represent the minimum and maximum (excluding outliers), and

the dots represent outliers. Asterisks indicate significant difference between LC-MS and WQA concentrations by the Wilcoxon rank sum test, at $p > 0.05$ (ns), $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) and $p < 0.0001$ (****). Red dashed lines represent the PNEC value for azoxystrobin, and blue dashed lines represent the AA and MAC EQS for triclosan. Triclosan: WQA $n = 76$ (2019), 3 (2020), 34 (2021), 83 (2022), and 4 (2023); LC-MS $n = 252$ (2019), 62 (2020), 183 (2021), 313 (2022), and 47 (2023). Azoxystrobin: WQA $n = 14$ (2021), 8 (2022), and 50 (2023); LC-MS $n = 297$ (2021), 470 (2022), and 95 (2023). Data used were >LoD only.

3.7. Multi-Data Comparison

Figure 6 illustrates a comparison of the three sources of riverine data with concentrations in treated effluent for six fungicides. The data depicted are from 2021 only, as substantially more effluent data were collected (and found to be >LOD) under the UKWIR CIP that year than any other. The higher concentrations associated with GC-MS relative to LC-MS are clearly evident in each case and notably exceed those of the WQA for azoxystrobin, but other patterns appear to be more fungicide-specific. The levels of triclosan in treated effluent show wide variation but are broadly higher than the riverine concentrations illustrated through the LC-MS and WQA data. This is in line with the predominant use of triclosan in consumer products, leading to its discharge to the sewer system. Hence, higher effluent concentrations are to be expected, with dilution then occurring in-river. Effluent concentrations of azoxystrobin, fludioxonil, and propiconazole are all lower than those under GC-MS, probably reflecting their predominantly agricultural use, whereby their wash-off to rivers would bypass the sewer network. However, they are all present in effluent, potentially reflecting amenity use where they are washed off to a combined sewer system, domestic use in homes and gardens, and residues on foodstuffs including fruits and vegetables [57]. The relatively high effluent concentration of tebuconazole, also used within agriculture, appears to be an outlier and may reflect its use as a preservative for wood and construction products. Effluent concentrations of epoxiconazole are relatively high despite its use being banned in 2021.

3.8. Triclosan in Sewage Sludge Applied to Land

Analysis of the UKWIR CIP dataset provided concentrations of triclosan in sewage sludge (data for other fungicides were not available) derived from 11 wastewater treatment plants in 2020 and 2021. Median levels, combined across each of the plants, were 1 mg/kg in 2020 and slightly lower in 2021 (Figure 7) and on the lower end of the range of those reported elsewhere [58]. They indicate that soil contamination is likely, together with the potential for subsequent transport to surface waters.

These sewage sludge concentrations are substantially higher than reported PNECs for triclosan for terrestrial organisms, which vary markedly, e.g., 0.06 mg/kg soil dry weight [59] and 0.196 mg/kg soil dry weight [60], but are lower than one reported microbial toxicity benchmark of 2 mg/kg [61]. While modeling approaches are described in the literature [61,62], harmonized methodologies for predicting soil and pore water fungicide concentrations arising from applications of sewage sludge are not available. This, coupled with the uncertainty around PNECs, limits the understanding of the risk posed by fungicides in sewage sludge applied to land.

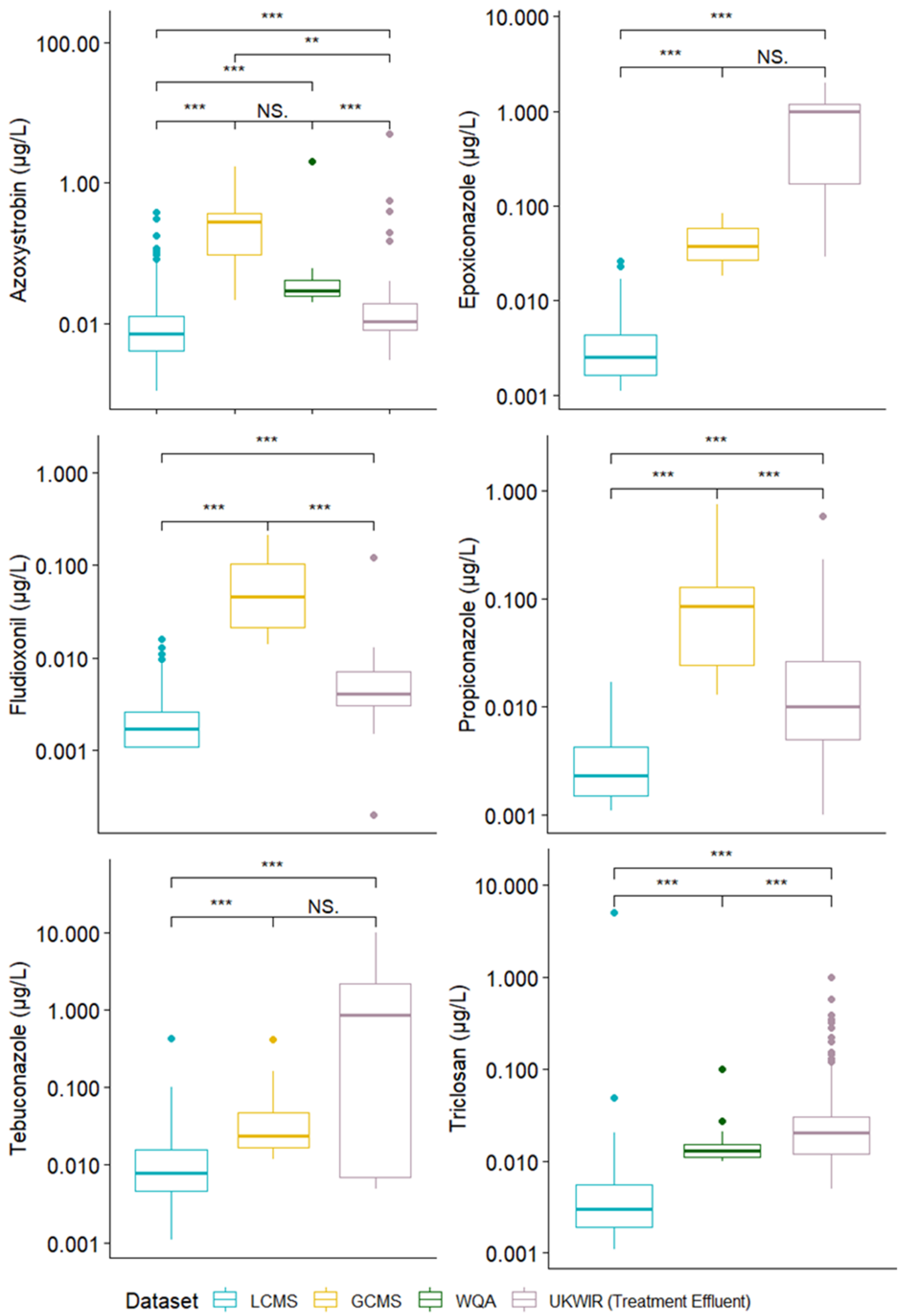

Figure 6. Boxplots of 2021 riverine concentrations from the LC-MS, GC-MS, and WQA datasets and 2021 treated effluent concentrations from the UKWIR-CIP for azoxystrobin, epoxiconazole, fludioxonil, propoconazole, tebuconazole, and triclosan. The boxes represent the upper and lower quartiles, the horizontal bars represent the median, the whiskers represent the minimum and maximum (excluding outliers), and the dots represent outliers. Asterisks indicate significant difference by the Wilcoxon rank sum test at, $p > 0.05$ (NS.), $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***). Azoxystrobin: $n = 5$ (GC-MS), 297 (LC-MS), 82 (UKWIR), and 14 (WQA); Epoxiconazole: $n = 6$ (GC-MS), 212 (LC-MS), and 5 (UKWIR); Fludioxonil: $n = 8$ (GC-MS), 65 (LC-MS), and 67 (UKWIR); Propiconazole: $n = 13$ (GC-MS), 195 (LC-MS), and 72 (UKWIR); Tebuconazole: $n = 36$ (GC-MS), 248 (LC-MS), and 12 (UKWIR); Triclosan: $n = 183$ (LC-MS),1127 (UKWIR), and 34 (WQA). Data used were >LoD only.

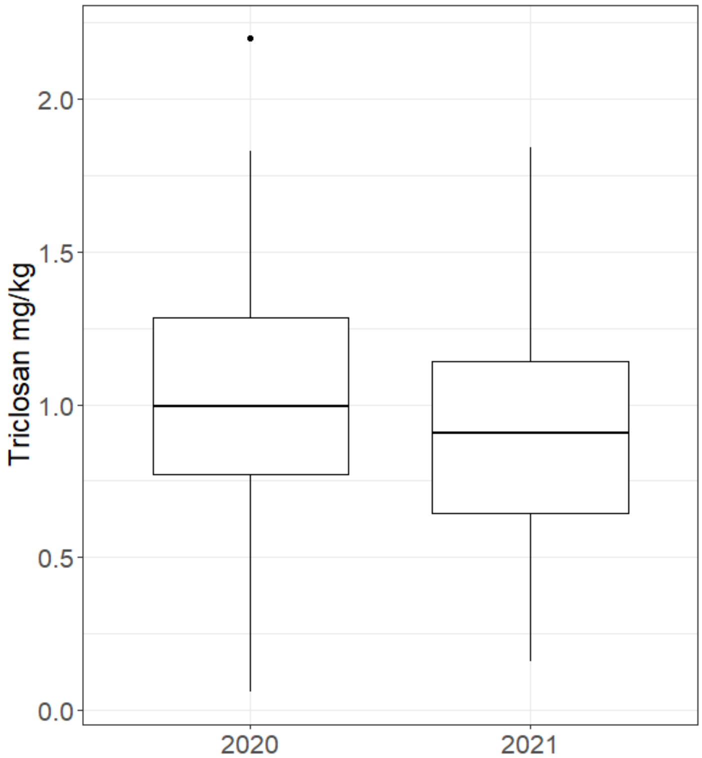

Figure 7. Boxplots portraying concentrations (mg/kg) of triclosan in sewage sludge in 2020 (n = 58) and 2021 (n = 144). The boxes represent the upper and lower quartiles, the horizontal bars represent the median, the whiskers represent the minimum and maximum (excluding outliers), and the dots represent outliers. Data used were >LoD only.

4. Discussion

Analysis of national datasets has shown fungicides to be widely detected in English rivers, with a prevalence broadly comparable to that found elsewhere [16,63]. While earlier studies have highlighted the potential importance of co-occurrence [16], the issue has not been widely examined. The observed co-occurrence in this study of several fungicides within the same sample and at the same site in English rivers provides strong evidence of the potential for synergistic mixture effects. It also adds weight to proposals for an accumulative approach to the risk assessment of azole fungicides due to their similar modes of action [26]. Moreover, the observed presence of fungicides with insecticides in combinations known to cause detrimental effects provides further evidence for the need to improve the monitoring of chemical mixtures and their combination effects [44]. The persistence of a banned fungicide, epoxiconazole, in this study suggests that risk assessment needs to include those fungicides now withdrawn from the market.

The semi-quantitative nature of the LC-MS and GC-MS datasets precludes a rigorous comparison of observed concentrations against predicted no effect concentrations; rather, the data lend themselves primarily to assessment of presence/absence. This, therefore, limits the understanding of the potential for detrimental impacts on aquatic biota arising from the widespread presence of fungicides in rivers. It also precludes assessment of the extent to which fungicides in the aquatic environment may be contributing to antifungal resistance through the exceedance of PNECs set for resistance, although the availability of such PNECs is, in any case, limited to only a few fungicides [64,65]. These information gaps need to be urgently addressed to enable a determination of the true risk that fungicidal compounds pose to the emergence of antifungal resistance.

To enable a fully comprehensive overview of ecotoxicological risk from fungicides in English rivers, several more need to be subject to a fully quantitative analysis rather than simply target screening alone (Table S1). Additionally, greater monitoring of fungicides used for domestic and amenity purposes is needed to better understand the relative importance of these sources and to balance the current disproportionate monitoring focus on fungicides used in agriculture. Monitoring programs also need to respond quickly to fungicides newly introduced to the market; mefentriflucuzole, for example, was introduced to the UK market in 2019 [41] and increased in use by up to 755% by 2022 [66]. These monitoring recommendations may have wider applicability. For example, several azole fungicides lacked sufficient or representative monitoring data to perform European Union-wide risk assessment when shortlisting substances for the Third Watchlist of the EU WFD [26].

One antifungal agent, triclosan, is subject to regulatory monitoring in England, and hence, a fully quantitative analysis is available whereby concentrations can be compared against an associated proposed EQS. Fully quantitative data are also provided for the fungicide azoxystrobin, whereby comparison against a proposed PNEC is possible. Exceedance of the AA EQS for triclosan and the proposed PNEC for azoxystrobin is rare. MAC exceedances for triclosan are more prevalent and raise implications for aquatic health at certain times and in certain locations. The impact of such exceedances may be exacerbated by co-occurrence with other fungicides.

A monthly spot sampling regime, at best, has been adopted for the monitoring of fungicides in English rivers. Such a temporal sampling frequency will infrequently capture high flow events [67] and therefore under-represent the detection of fungicides washed to rivers via rainfall-driven processes, including runoff from agricultural and amenity land, and storm overflows. Concentrations during and immediately following storm events can be substantially higher than those at base or mean flow [16,68]. Sampling during periods of high flow is therefore necessary to capture peak fungicide concentrations and improve the understanding of the ecotoxicological impact that they may have. Passive samplers can be deployed in this regard [10] to complement spot sampling.

Some patterns in monthly variation are discernible for selected fungicides. More detailed analysis of this issue may help to improve the temporal targeting of sampling, enabling the focusing of greater resources on certain fungicides during key times of the year. To support this, improved spatial and temporal information on fungicide usage is required [69], with the United Kingdom's Food and Environment Research Agency's pesticide usage survey covering only 6% of the total area of arable crops grown [70]. No fungicides sales data are available either.

The concentrations of triclosan in sewage sludge examined in this study suggest the potential for deleterious effects on soil ecosystems following application to land. While the fate of fungicides in wastewater treatment plants is variable, some show good removal and sorption to sewage sludge [71]. The risk that may be posed by fungicides applied to land within sewage sludge remains a clear knowledge gap. Addressing this requires the quantification of the range of fungicides found in sewage sludge and the frequency of application to land, quantification of degradation rates, understanding of the partitioning of fungicides between soil and pore water, and the development of harmonized soil and microbial PNECs.

The potential for fungicides to adsorb to sediments and organic surfaces in aquatic systems [10,58,72], coupled with the observed co-occurrence of fungicides in this study, suggests that several fungicides may be present together in riverbed sediments, at least in the more contaminated locations. For example, azoxystrobin, shown to occur widely in this study, is rapidly lost to sediment sorption when entering waterways [19,54]. This raises implications for the health of benthic biota and suggests that monitoring programs should extend beyond monitoring the water column to also address bed sediments. This aligns with proposed changes in regulatory approaches, whereby the Third and Fourth Watch Lists for the EU Water Framework Directive identified the need to include sediment monitoring for hydrophobic compounds [26,45]. Given the emerging threat of antimicrobial resistance, extending this approach to biocidal compounds is recommended.

5. Conclusions

Fungicides are prevalent in English rivers, and co-occurrence occurs widely with up to nine different fungicides detected within the same sample, raising concerns for synergistic interactions. The semi-quantitative nature of much of the available data precludes a clear determination of the potential risk of detrimental effects on aquatic biota. Fully quantitative analysis is required, and ecotoxicity-based water quality standards need to be agreed upon. The monthly sampling regime needs to be complemented with monitoring during high flow events to capture those fungicides transported to rivers via rainfall-driven processes. Monitoring should also extend beyond the water column to address fungicides in riverbed sediment. Several information gaps exist including the risk posed by fungicides in sewage sludge applied to land and the extent to which fungicides in the aquatic and terrestrial environments contribute to antifungal resistance. Improvements in spatial and temporal information on fungicide sales and use are needed.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/environments12020045/s1, Table S1: Visualisation of fungicides reported in each database from 2019–2023 inclusive. Green = reported, orange = present on determinand list but either not reported or below detection limit, red = not monitored (to the best of our knowledge from the determinand lists available).

Author Contributions: N.P.: Investigation, Visualization, Formal analysis, Writing—original draft, Writing—review and editing. R.C.: Conceptualization, Formal analysis, Writing—original draft, Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: All data used in this work are publicly available for download at the following sources: Water Quality Archive, Defra: https://environment.data.gov.uk/water-quality/view/landing (accessed on 26 February 2024); Water quality monitoring data GC-MS and LC-MS semi-quantitative screen-data.gov.uk, Environment Agency: https://www.data.gov.uk/dataset/0c63b33e-0e34-45bb-a779-16a8c3a4b3f7/water-quality-monitoring-data-gc-ms-and-lc-ms-semi-quantitative-screen#:~:text=The%20Environment%20Agency%20uses%20semi,range%20of%20substances%20at%20once (accessed on 13 February 2024); Chemical Investigations Programme, UKWIR: https://ukwir.org/leading-the-water-industry-research-agenda (accessed on 28 February 2024).

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