Use of Chemcatcher® passive sampler with high-resolution mass spectrometry and multi-variate analysis for targeted screening of emerging pesticides in water

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Abstract

Pesticides present at trace concentrations are a common cause of poor water quality. Their concentrations can change dynamically, due to the stochastic nature of pesticide pollution. Consequently, characterisation of pesticide residues that are intermittently present, poses significant monitoring and analytical challenges. Traditional approaches rely on quantitation of a limited number of pesticides present in a discrete water sample. Expanding the analytical suite and/or the frequency of sampling to meet these challenges is often impractical. Comprehensive methods are needed, with selectivity and sensitivity for the hundreds of pesticides potentially present, and temporal representativeness to ensure changing conditions are understood, in order to identify and prioritise risk. Recent analytical advances have enabled the targeted screening of hundreds of compounds in the same run, and automated work-flows can now reliably identify compounds through the comparison of retention time and accurate mass with spectral libraries. Screening generates large qualitative data sets, therefore, there is a need for improved monitoring methods and data interpretation strategies to reduce the need for repetition, and increase the quality of information for end-users. Passive sampling is an in-situ time integrative technique, increasingly used for monitoring pesticides in water. Here, we describe a method using the Chemcatcher® passive sampler, coupled to targeted screening using liquid chromatography-quadrupole-time-of-flight mass spectrometry, and a commercially available library. Statistical analysis was performed using Agilent Mass Profiler Professional software. Water sampling took place over one year, at three riverine sites in the south of England, UK. Statistical interpretation of time integrative data from passive sampling could distinguish regular and episodic pesticide inputs, and detected compounds neglected by routine monitoring methods. One hundred and eleven pesticides were identified including legacy and current use compounds with diverse origins and uses. Spatial and temporal trends were identified enabling prioritisation of seasonal monitoring at each site. This approach maximises the utility of qualitative assessment and may help water quality managers to rationalise pesticide fate in future, providing significant additional insight without the need to increase the scope and cost of monitoring.

Introduction

Surface waters are often contaminated by complex mixtures of chemicals present at trace concentrations. These originate from diverse sources and identifying constituents of such mixtures is a priority of current research.[1] One class of contaminants; pesticides, are heavily used throughout the

world.[2] Pesticides are products that prevent damage caused by pests such as, insects, weeds and fungi.[3] The largest source of pesticides is agriculture, where plant protection products (PPPs) are applied to most agricultural land. To meet growing demand for food, pesticide usage is set to increase until 2050, in line with past trends that have seen a 20-fold increase in use since 1960.[4] Pesticides are used in other applications such as public heath, veterinary medicine, household, and industry.[3] Approvals of novel compounds and banning of existing compounds causes changes in use over time.[5] This results in an expanding list of current use and legacy compounds; for example the pesticide database of the European Union pesticides contains ~ 1300 compounds, of which less than half have approval.[6]

Polar compound chemistries with a lower potential for bioaccumulation and persistence are favoured in many modern pesticides. However, polar pesticides can preferentially move to the aquatic environment through several pathways.[7] Differences in mobility and environmental persistence between pesticides combine with temporal and spatial variation in usage and landscape processes to produce highly fluctuating concentrations in water.[8–10] Many analytical techniques can detect aqueous pesticides at trace concentrations (ng L^{-1} to µg L^{-1}), and pesticides are widely observed in environmental waters. However, knowledge of pesticide fate is limited, and the risk to humans and ecosystems is not well understood.[11]

Awareness of the need to characterise the risk posed by polar pesticides is increasing, and several are included in the list of priority substances (e.g. isoproturon), for monitoring under the European Union Water Framework Directive (WFD).[12] Comparable monitoring programmes for pesticides exist in only 26% of jurisdictions globally.[13] Long-term monitoring is important in understanding temporal trends. This understanding can be improved by increasing the number of sampling sites or sampling frequency; however, this is often impracticable on the grounds of cost. Routine monitoring programmes use discrete, low volume spot samples, coupled to targeted analysis.[1] This provides a 'snapshot' at the time of sampling and does not accurately capture variation over time and may miss pesticides present that are outside the analytical measurement suite.[14] Furthermore, to achieve required limits of quantification (LOQ) or detection (LOD) for all pesticides in a sample, large volumes of water and/or several sample clean-up and enrichment steps may be required.[15]

Passive sampling is an alternative monitoring method. Here freely dissolved analytes present in sampled waters are sequestered in a receiving phase within a device.[16] This time-integrative method overcomes several limitations associated with spot (bottle or grab) water sampling; achieving higher sensitivity and being more temporally representative, without increasing the frequency of sampling.[1] Passive sampling device (PSD) design, configuration, and operation can be altered to achieve sensitivity and selectivity for different analyte classes, concentrations and exposure periods.[17] Uptake into devices operated in the kinetic regime is proportional to changes in ambient concentrations, allowing measurement of time-weighted average (TWA) concentrations. Alternatively, equilibrium concentrations can be obtained if the PSD is allowed to equilibrate with the sampled waters.[5] It is usually only appropriate to operate PSDs in the equilibrium regime for non-polar analytes.[16] Equilibrium sampling of polar analytes carries higher uncertainty, the causes of which are not currently well understood.[2] A variety of PSDs has been developed to monitor polar pesticides, with most devices developed to monitor in the kinetic regime.[2,17] Recent reviews provide a comprehensive overview of PSD operation and theory for organic analytes under equilibrium and kinetic, sampling regimes.[2,16] Sampling rates in PSDs are analyte specific, and devices require calibration before TWA or equilibrium concentrations can be derived from the sampled mass of analyte.[18] Alternatively devices can be deployed in qualitative assessments, for example, in suspect screening or non-target screening

approaches.[19–21] Devices with selectivity and sensitivity for monitored analytes must be coupled to analytical methods to which monitored analytes are similarly amenable.

High performance analytical methods for chromatographic separation and mass measurement (e.g. quadrupole-time-of-flight mass spectrometry (Q-TOF) and Orbitraps) are routinely applied to environmental samples allowing detection of large numbers of analytes in a single analytical run.[22] The increasing availability of commercial suspect compound databases, compatible analytical instruments and automated post-acquisition processing, has simplified suspect screening workflows allowing rapid generation of a tentative suspect list. Various strategies have been developed to filter this tentative list to a final suspect list, by determining false positives and negatives through manual comparison with analytical parameters and control samples.[23]

This study presents a standardised method for identifying high consequence pesticides and prioritising these for future monitoring from qualitative analytical data, using a simple and reproducible approach. We aimed to increase the representativeness of monitoring by applying time-integrative sampling coupled to comprehensive screening, within the framework of a long-term monitoring programme at three sites in a river catchment in South East England. This qualitative approach was designed to capture information neglected by conventional approaches without the need to increase the scope of monitoring. Through use of a range of multivariate statistics we reduced data complexity and identified spatial and temporal tends in the occurrence and abundance of monitored pesticides. Risk at a potable water abstraction was then prioritised in terms of seasonal and spatial variation in sources of pollution within the catchment. A seasonal monitoring programme at each site, informed by the prioritised risk at the potable water abstraction is presented.

Materials and methods

Chemicals, glassware and reagents

All solvents were obtained from ThermoFisher Scientific (Loughborough, Leicestershire, UK) and were of HPLC-grade or better. Ultra-pure water (UPW) (> 18.0 MΩ•cm @ 25°C) was produced using an in-house Milli-Q® purification system (Merck, Burlington, USA). Formic acid was purchased from Sigma-Aldrich (Dorset, UK). UPW was used in all laboratory procedures. Glassware was soaked in a 5% Decon 90 solution (Decon Laboratories Ltd, Hove, UK) overnight and rinsed with UPW then methanol (MeOH) before use.

Chemcatcher® passive sampler preparation

The Chemcatcher® (Atlantic version [ESI Fig. S1]) comprising a polytetrafluoroethylene (PTFE) base and retaining ring was obtained from AT Engineering (Tadley, UK). Prior to use, Chemcatcher® components were cleaned with a brush in lukewarm water then soaked in a 5% Decon 90 solution for 24 h. Components were then rinsed in UPW and allowed to dry at room temperature, before being immersed for 10 min in an ultrasonic bath containing acetone. Components were then rinsed with UPW and allowed to dry at room temperature until assembly. The sampling disk used was a hydrophilic-lipophilic balanced 47mm Horizon Atlantic™ SPE disk (HLB-L) (Biotage, Uppsala, Sweden) which has a high affinity for analytes over a broad polarity range.

Acetone rinsed stainless steel tweezers were used to handle delicate consumables and to avoid contamination. HLB-L disks were washed by soaking in MeOH overnight. Disks were then conditioned in an extraction manifold under gentle vacuum with MeOH (50 mL) followed by HPLC grade water (100 mL), ensuring disks did not dry out. Disks were then left submerged in water until assembly.

Polyethersulfone (PES) (Supor® 200, 0.2 µm pore diameter) sheet, obtained from Pall Europe Ltd (Portsmouth, UK) was used as diffusion membrane. Each discrete circular membrane (52 mm diameter) was punched from the PES sheet. Membranes were then rinsed five times in MeOH, soaked overnight in MeOH, rinsed once more in MeOH followed by twice in UPW and stored in UPW until assembly. This step was necessary to remove oligomer artefacts (polyethylene glycol) resulting from manufacture, which cause matrix effects during instrumental analysis and has been adapted from Guibal et al.[24] Chemcatcher® PSDs were then assembled by placing the flat side of the HLB-L disk onto the base plate and overlaying this with the membrane, ensuring the circumference of each were aligned. The retaining ring was then used to secure each in place, taking care to avoid air pockets in the interstitial space and over tightening, whilst achieving a watertight seal. Assembled Chemcatcher® PSDs were then submerged in UPW in a sealed polyethylene terephthalate (PET) container and stored at 4°C until deployment. The PET containers were soaked in 5% Decon 90 solution overnight and rinsed with UPW prior to use. This procedure has been reported previously by Castle et al.[8]

Sampler deployments

Chemcatcher® PSDs were deployed at three sites on the River Arun within the Arun and Western Rother river catchment in South East England (Fig. 1) (see ESI for a full description of the study area). This area is used for the capitation of potable water[25] and has diverse hydrology, land use, and pollution sources. Land use within the catchment is primarily arable or pasture, with scattered urban conurbations (e.g. Horsham, Petersfield, Midhurst and Pulborough), industry, woodland, meadow and amenity grassland.[26,27] Site 1 was located in the upper reaches of the catchment immediately downstream of a large wastewater treatment works, and sites 2 and 3 were located approximately 0.5 km before and after the confluence with the Rother, respectively. Site 3 was also the location of a surface water abstraction for potable supplies.

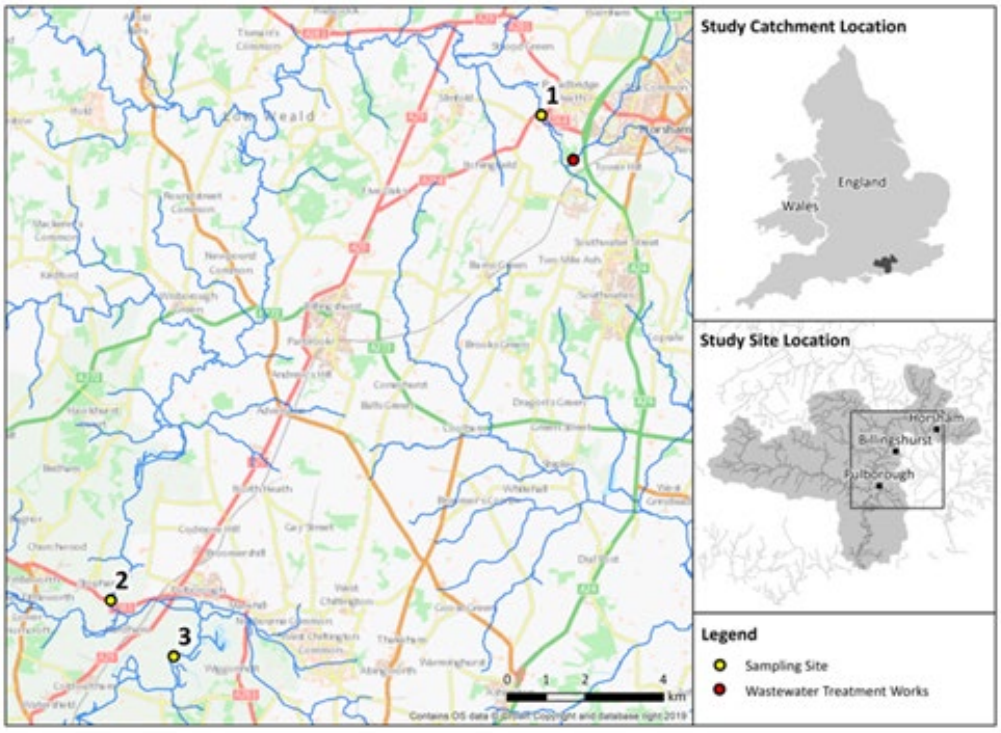

Fig. 1 The three sampling locations in the Arun and Western Rother catchment, in South East England.

Sites were chosen to be representative of different land use, and likely pollution sources along the length of the Arun. Sampler deployments occurred at two-week intervals over twelve months (October 2017 to October 2018), totalling 25 deployments. This was to ensure data were inclusive of any seasonal variation in usage, vectors, hydrology and degradation, which influence pesticide presence and fate. PSD deployments occurred at sites that were also used for spot sampling by the local water utility. A deployment rig (ESI Fig. S2 and S3) was designed to allow samplers to be positioned in the water column, and subsequently retrieved. All three rig deployments were from bridges across the river.

Chemcatcher® PSDs were transported to and from the field in a sealed PET container inside a cool box. At each site two Chemcatcher® PSDs were removed from their container and placed into the deployment apparatus. After deployment Chemcatcher® PSDs were removed from the deployment apparatus, wrapped in aluminium foil and placed in a labelled zip lock bag and transported to the laboratory in a cool box and maintained at ~ 4 °C until analysis, which usually occurred within a week. At the end of each deployment two PSDs were isolated and used as field blanks.

Chemcatcher® passive sampler extraction

Exposed and blank Chemcatcher® PSDs were disassembled in the laboratory. The membrane discarded and the HLB-L disk placed carefully on MeOH rinsed aluminium foil and dried at room temperature. HLB-L disks were then stored at -18°C prior to extraction. Only one HLB-L disk per deployment and one field blank were extracted. The other disk was used a back-up sample. Prior to extraction, HLB-L disks were allowed to reach room temperature. HLB-L disks were then placed in an extraction manifold and eluted under gravity with MeOH (40 mL) into a glass screw top vial (60 mL). One mL of HPLC grade water was then added to each vial as an analyte retainer. The eluent was then evaporated to ~ 0.5 mL in a Genevac EZ-2 centrifugal rotary evaporator (Genevac Ltd, Ipswich, UK) set at 40°C. Extracts were then transferred to 2 mL deactivated (silanized) vials (Agilent, Santa Clara, USA), adjusted to 1 mL with MeOH, weighed, then stored at -18°C prior to instrumental analysis.

Chemcatcher® PSDs were prepared in a single batch before every deployment. To ensure quality assurance and control, solvent, production and field blanks were produced to identify contamination during conditioning, assembly and field handling, extraction and instrumental analysis. One solvent blank consisting of a HLB-L disk, isolated immediately after conditioning, and one production blank, consisting of an assembled Chemcatcher® PSDs isolated after assembly were included per batch. Each batch was extracted and analysed concurrently.

Instrumentation and software

Chromatographic separation was achieved with a Dionex Ultimate 3000 UHPLC system containing a Dionex Acclaim RSLC 120 C_{18} analytical column (2.1 i.d. × 100 mm length, 2.2 µm particle size), (Thermo Fisher Scientific, Bremen, Germany) and a VanGuard, Acquity UPLC BEH C_{18} guard column (1.7 µm particle size), (Waters, Dublin, Ireland). Mass spectrometry was undertaken with a Bruker Maxis Impact II electrospray high resolution time-of-flight tandem mass spectrometer (Q-TOF-MS) (Bruker Daltonics, Bremen, Germany), with the following operating parameters: capillary voltage, 2500 V; end plate offset, 500 V; nebulizer pressure, 2 bar (N_2); drying gas, 8 L min^{-1} (N_2); and drying temperature, 200 °C. Data acquisition used Bruker HyStar acquisition software (rev. 3.2) and data interpretation (analyte identification and quantitation) used Bruker Target Analysis for Screening and

Quantitation (TASQ®) 1.4 software. Software and hardware used for chromatographic separation, mass spectrometry, data acquisition and data interpretation where interfaced and used to screen against Bruker's PesticideScreener™ (2.1) database. Statistical interpretation of identified analytes was undertaken after transferring outputs contained in TASQ® into Mass Profiler Professional (MPP) software (B.14.9.1) (Agilent, Santa Clara, USA).

Instrumental analysis

Prior to each chromatographic run an automatic mass axis calibration was undertaken (lock mass calibration was not used). A syringe pump introduced the calibrant solution (1 mM sodium formate in water/isopropanol/formic acid (1:1:0.01 v/v/v)) into the mass spectrometer before analyte elution from the analytical column. Mobile phase A was an aqueous solution of 10% of MeOH, 5 mM ammonium formate and 0.01% v/v formic acid. Mobile phase B was MeOH with 5 mM ammonium formate and 0.01% v/v formic acid. Extracts were diluted (1:9 v/v) in mobile phase A and 20 µL was then injected into the column, which was maintained at 30°C. The gradient and flow elution programme was: 0 min, 1% B, 0.2 mL min^{-1}; 3 min, 39% B, 0.2 mL min^{-1}; 14 min, 99.9% B, 0.4 mL min^{-1}; 16 min, 99.9% B, 0.48 mL min^{-1}; 16.1 min, 1% B, 0.48 mL min^{-1}; 19.1 min, 1% B, 0.2 mL min^{-1}; and 20 min, 1% B, 0.2 mL min^{-1}.

The Q-TOF-MS was operated in the broadband collision-induced dissociation (bbCID) acquisition mode and data were collected between 0.8 and 15.0 min. bbCID data acquisition ensures all compounds eluting from the analytical column and amenable to ionisation are captured all of the time, facilitating retrospective analysis of unknowns. Spectra were recorded at a scan rate of 2 Hz and scan range of m/z 30-1000. The bbCID mode generated full-scan MS and MS/MS spectra consecutively by alternating between a low collision energy of 6 eV (MS) and a ramped high collision energy 30 eV +/- 6 eV (MS/MS).

Filtering procedure

TASQ® identified target analytes through automated comparison of extracted ion chromatograms (including molecular ions, protonated and sodiated adduct ions and associated fragment ions) with theoretical values for mass accuracy (± 5 ppm) and retention time (± 0.5 min) with a signal to noise ratio < 3. Manual verification of this preliminary list was performed to increase the confidence of identification. Positive identification required isotopic fit < 250 mSigma with a peak abundance > 5,000 and was qualified by the presence and relative peak intensity of diagnostic ions (MS/MS fragment ions). Positive identification was made if at least one fragment ion including the precursor ion (typically the protonated molecular adduct) were present in the extracted ion chromatograms. Where fragment ions were not present, identification only required the precursor ion and first isotope but was made with a lower degree of confidence. Analytes present in field, production or solvent blanks, or the analytical mobile phase were manually removed unless they were significantly and consistently (a minimum of three times) higher in the field sample extracts. This workflow was optimised to reduce false positives and was adopted because the PesticideScreener™ database contained many of the polar pesticides for which the version of the Chemcatcher® used has high affinity (selectivity and sensitivity) for. There are exceptions, however, for which the analytical method exhibited poor sensitivity for certain compounds e.g. metaldehyde. Any false negatives obtained were checked by looking at each of the compounds extracted ion chromatograms to check for correct retention time, accurate mass against theoretical mass, peak area response and peak symmetry, and ion ratio. The

PesticideScreener™ contained up to seven diagnostic ions for an associated precursor ion, however, for most analytes fewer diagnostic ions were available. Diagnostic ions with a relative intensity > 50% of the precursor ion are marked as mandatory. To be eligible as a diagnostic ion a fragment ion must have a relative intensity of > 5% of the most abundant fragment ion. For a number of analytes no diagnostic ion was available due to low fragmentation efficiency. An outline of this workflow including screening and scoring of identification confidence is given in Fig. 2. Instrumental analysis was undertaken in triplicate in positive ionisation mode. Selected samples were also analysed in negative ionisation mode to see if any compounds that had a weak response in positive ion mode would be more readily detected/identified in negative ion. No advantage, in reliable detectability was observed in negative ion mode and only positive ionisation mode data is included for brevity.

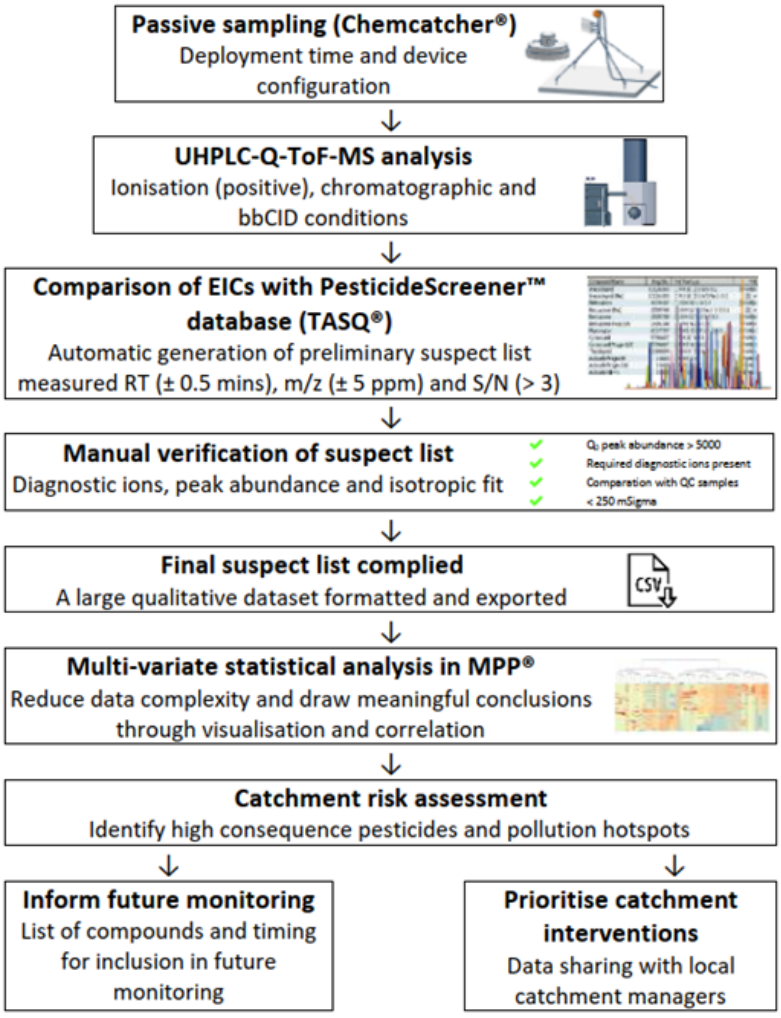

Fig. 2 Workflow for monitoring, instrumental and statistical analysis and catchment risk assessment. EICs: extracted ion chromatograms.

Multi-variate (statistical) analysis

Multivariate analysis was performed in Agilent Mass Profiler Professional (MPP, B.14.9.1). Data were imported in a generic format and peak abundance was Z-transformed to normalise data using the equation below:

$$C_j = \frac{(N_j - M_i)}{S_i}$$

- A compound *C* has normalised abundance N_1, N_2, ….. N_n,

(n = number of samples)

- C_j = baselined value for compound in j^{th} sample
- M_i = mean intensity value across all samples
- S_i = standard deviation value across all samples

This was performed to reduce the influence of instrumental stability, matrix effects and sampling rate limitation due to ambient conditions on the instrument response over time and to allow comparison of trends data. Data on occurrence and normalised peak abundance were used to produce Venn diagrams, box and whisker and hierarchical cluster analyses. Hierarchical clustering analysis (HCA) was applied to all samples to elucidate groupings within variables (analytes) and conditions (deployments). A mean value for each analyte and deployment was used. Interval measures for clustering, within, and between, groups used a Euclidean distance metric and Wards linkage method. HCA grouped clusters of analytes based on similarity and dissimilarity in the data set. Clustering was performed on analytes and conditions to interpret temporal trends for related analytes. A mean value for each analyte and season (multiple deployments) was computed to produce box and whisker diagrams for each cluster identified in the HCA. Venn diagrams were employed to assign each analyte a code according to site occurrence.

Risk assessment and actions Detection frequency at site 3 was used to estimate risk to the water supply works (WSW) abstraction at the site. This stepwise method using the outputs of these analyses was used to design future monitoring within the catchment. Analytes present within one cluster were characterised to identify probably sources of pollution and mitigation and monitoring strategies.

Results and discussion

Passive sampling

All PSDs were successfully recovered at the end of each deployment. The deployment rig was removed from the water in one instance (deployment 14, site 2), however, devices were found to be intact and have been included in the presented data. Site 2 was inaccessible for a week at the beginning of deployment 8. Chemcatcher® devices remained in place over this time and were later retrieved. To accommodate this interruption deployments 7 and 9 both took place consecutively, each lasting 3 weeks, and deployment 8 is omitted from the data.

The level of fouling of devices was consistent between each site but varied from deployment to deployment. Fouling and other environmental factors such as flow velocity and temperature can

influence analyte uptake into passive sampling devices.[16] To account for the influence of environmental conditions, laboratory and/or *in-situ* calibration experiments are typically performed for each analyte.[18] Continuous monitoring of flow and physiochemical parameters at fixed monitoring stations throughout the catchment were checked. All sites experienced similar changes in relative environmental conditions throughout monitoring. No attempt to account for site specific environmental conditions was made, as it was not expected to affect the qualitative data obtained from the trial.

Targeted screening and confirmation

Field, production and solvent blank PSDs were analysed for each deployment resulting in tentative identification of 30 analytes. Details of blank PSDs and solvent samples are presented in Table S1.

After manual verification of the suspect list 15 analytes present in blank and solvent samples were removed to reduce the possibility of false positives. Three analytes detected in blank and solvent samples were not removed as relative peak abundance in environment indicated this may have resulted in false negatives. The remaining analytes detected in blank and solvent samples were absent in environmental samples. Table S2 lists the analytes present in solvent and blank samples and their treatment during manual verification.

The suspect list database (Bruker PesticideScreener™ database) contained 848 pesticide compounds. Filtering of the initial suspect list reduced the suspect list to 113 analytes. The analytical method was unable to distinguish benomyl from its metabolite carbendazim and these are presented jointly in the data. The obsolete triazine herbicide sebutylazine was always detected alongside terbuthylazine at approximately half the relative peak abundance. A collaborative trial screened environmental water samples using a range of instruments, experimental conditions and filtering criteria, but could not distinguish between sebutylazine and terbuthylazine owing to their isobaric nature (i.e. co-elution and molecular adduct ions (plus fragment ions) with the same masses).[29] As sebutylazine is not currently approved for use it was manually removed from the suspect list and only terbuthylazine assumed to be present. The final suspect list contained 111 analytes. This number included several instances of chiral compounds sharing the same peak and compounds where detection was possible based on multiple peaks, in the extracted ion chromatogram (EIC). Seven analytes were tentatively identified where the precursor ion was a fragment ion with the remainder of the tentatively identified analytes made with an adduct of the molecular ion.

Identified pesticides

One hundred and eleven analytes were detected across all sites and deployments. These are shown in Table 1.

Table 1 Experimental conditions and number of detections of tentatively identified polar pesticides with Chemcatcher® PSDs during 25 consecutive deployments at three sites on the River Arun occurring over one year.

Compound*	Type**	Elemental Composition	CAS Number	Precursor ion	Theoretical m/z	Theoretical RT [min]	Site 1	Site 2	Site 3	Total	% Detec
1.2.3.6-Tetrahydrophthalimide (cis-)	F	$C_8H_{10}NO_2^{1+}$	(1469-48-3)	M+nH	152.0706	4.37	18	17	24	59	80
1-Naphthylaceticacid (NH4)	PGR	$C_{12}H_{14}NO_2^{1+}$	(86-87-3)	I	204.1019	7.76	2			2	3
2-hydroxyterbuthylazine	Me	$C_9H_{18}N_5O^{1+}$	(66753-07-9)	M+nH	212.1506	5.7	12	19	13	44	60
Aldicarb-sulfone (Aldoxycarb) Fragm 148	I, N, Me	$C_5H_{10}NO_2S^{1+}$	(1646-88-4)	I	148.0427	3.75	6	1		7	10
Allethrin I	I	$C_{19}H_{27}O_3^{1+}$	(584-79-2)	M+nH	303.1955	12.14	6			6	8
Atrazine	H	$C_8H_{15}ClN_5^{1+}$	(1912-24-9)	M+nH	216.101	8.16		5	4	9	12
Atrazine 2-Hydroxy	Me	$C_8H_{16}N_5O^{1+}$	(2163-68-0)	M+nH	198.1349	5.03	4	1		5	7
Atrazine-desethyl	Me	$C_6H_{11}ClN_5^{1+}$	(6190-65-4)	M+nH	188.0697	5.73			1	1	1.4
Azoxystrobin	F	$C_{22}H_{18}N_3O_5^{1+}$	(131860-33-8)	M+nH	404.1241	9.02	24	13	17	54	73
Bendiocarb	I, Ac, VS	$C_{11}H_{14}NO_4^{1+}$	(22781-23-3)	M+nH	224.0917	7.02	1	2	21	24	32
Benomyl (decomposed to Carbendazim) Fragm 192	F	$C_9H_{10}N_3O_2^{1+}$	(17804-35-2)	I	192.0768	5.46	5	2		7	10
Bixafen	F	$C_{18}H_{13}Cl_2F_3N_3O^{1+}$	(581809-46-3)	M+nH	414.0382	10.59	4	1	1	6	8
Boscalid	F	$C_{18}H_{13}Cl_2N_2O^{1+}$	(188425-85-6)	M+nH	343.0399	9.4	4	3	5	12	16
Bromacil	H	$C_9H_{14}BrN_2O_2^{1+}$	(314-40-9)	M+nH	261.0233	7.07	3			3	4
BTS 40348 (metabolite prochloraz)	Me	$C_{11}H_{15}Cl_3NO^{1+}$	(67747-01-7)	M+nH	282.0214	7.1			6	6	8
BTS 44595 (metabolite prochloraz)	Me	$C_{12}H_{16}Cl_3N_2O_2^{1+}$	(139520-94-8)	M+nH	325.0272	11.19	1	7	17	25	34
Carbetamide Fragm 192	H	$C_{10}H_{10}NO_3^{1+}$	(16118-49-3)	I	192.0655	6.58		4	3	7	9.5
Carbofuran 3-keto-	I, N, Ac, Me	$C_{12}H_{14}NO_4^{1+}$	(16709-30-1)	M+nH	236.0917	5.94	1	1	1	3	4.1
Carbofuran-3-hydroxy	I, N, Ac, Me	$C_{12}H_{16}NO_4^{1+}$	(16655-82-6)	M+nH	238.1074	5.09	1	1		2	2.7
CGA 321113 (Trifloxystrobin Metabolite)	Me	$C_{19}H_{18}F_3N_2O_4^{1+}$	(252913-85-2)	M+nH	395.1213	10.13	6	6	4	16	21.6
Chlorotoluron	H	$C_{10}H_{14}ClN_2O^{1+}$	(15545-48-9)	M+nH	213.0789	7.98		5	5	10	13.5
Cinosulfuron	H	$C_{15}H_{20}N_5O_7S^{1+}$	(94593-91-6)	M+nH	414.1078	6.36	1			1	1.4
Climbazole	F	$C_{15}H_{18}ClN_2O_2^{1+}$	(38083-17-9)	M+nH	293.1051	9.88	2			2	2.7
Clomazone (Command)	H	$C_{12}H_{15}ClNO_2^{1+}$	(81777-89-1)	M+nH	240.0786	8.89	5	15	18	38	51.4
Clothiandin	I	$C_6H_9ClN_5O_2S^{1+}$	(210880-92-5)	M+nH	250.016	4.9	17	18	24	59	79.7
Coumatetralyl	Ro	$C_{19}H_{17}O_3^{1+}$	(5836-29-3)	M+nH	293.1172	9.35	1			1	1.4
Cycloheximide Peak 1	F	$C_{15}H_{24}NO_4^{1+}$	(66-81-9)	M+nH	282.17	5.99	1			1	1.4
Cycloxydim II	H	$C_{17}H_{28}NO_3S^{1+}$	(101205-02-1)	M+nH	326.1784	11.76	2			2	2.7
Cycluron	H	$C_{11}H_{23}N_2O^{1+}$	(2163-69-1)	M+nH	199.1805	8.47	4	6	2	12	16.2
Cyproconazole Peak 1	F	$C_{15}H_{19}ClN_3O^{1+}$	(94361-06-5)	M+nH	292.1211	9.69	10	16	16	42	56.8
Cyproconazole Peak 2	F	$C_{15}H_{19}ClN_3O^{1+}$	(94361-06-5)	M+nH	292.1211	10.01	11	15	15	41	55.4
Cyromazine	I	$C_6H_{11}N_6^{1+}$	(66215-27-8)	M+nH	167.104	2.78	2	1		3	4.1
DCPMU (1-(3.4-DICHLOROPHENYL)-3-METHYL UREA)	H	$C_8H_9Cl_2N_2O^{1+}$	(3567-62-2)	M+nH	219.0086	8.22	20	7		27	36.5
DCPU (1.3.4-dichlorophenyl-urea)	H	$C_7H_7Cl_2N_2O^{1+}$	(2327-02-8)	M+nH	204.993	7.73	4			4	5.4
DEDIA (Desethyl-Deisopropyl-Atrazine)	Me	$C_3H_5ClN_5^{1+}$	(3397-62-4)	M+nH	146.0228	7.29	1			1	1.4
DEET (Diethyltoluamide)	I, Re	$C_{12}H_{18}NO^{1+}$	(134-62-3)	M+nH	192.1383	8.2	25	24	25	74	100.0
Diazinon	I, Re, VS	$C_{12}H_{22}N_2O_3PS^{1+}$	(333-41-5)	M+nH	305.1083	11.04	3			3	4.1
Dichlorobenzamide	Me	$C_7H_6Cl_2NO^{1+}$	(2008-58-4)	M+nH	189.9821	4.48	25	24	24	73	98.6
Dichlorvos	I, Ac, Me	$C_4H_8Cl_2O_4P^{1+}$	(62-73-7)	M+nH	220.9532	7		2	2	4	5.4
Diflufenican	H	$C_{19}H_{12}F_5N_2O_2^{1+}$	(83164-33-4)	M+nH	395.0813	11.72	2			2	2.7
Dimethenamid	H	$C_{12}H_{19}ClNO_2S^{1+}$	(87674-68-8)	M+nH	276.082	9.26		8	12	20	27.0
Dimethomorph Peak 1	F	$C_{21}H_{23}ClNO_4^{1+}$	(110488-70-5)	M+nH	388.131	9.22		1	1	2	2.7
Dimethomorph Peak 2	F	$C_{21}H_{23}ClNO_4^{1+}$	(110488-70-5)	M+nH	388.131	9.59		1	4	5	6.8
Dioxacarb	I	$C_{11}H_{14}NO_4^{1+}$	(6988-21-2)	M+nH	224.0917	5.16	1			1	1.4
Diuron	H	$C_9H_{11}Cl_2N_2O^{1+}$	(330-54-1)	M+nH	233.0243	8.55	24	24	25	73	98.6
Epoxiconazole	F	$C_{17}H_{14}ClFN_3O^{1+}$	(133855-98-8)	M+nH	330.0804	10.26	20	21	23	64	86.5
Fenamidone	F	$C_{17}H_{18}N_3OS^{1+}$	(161326-34-7)	M+nH	312.1165	9.22			1	1	1.4
Fenhexamid	F	$C_{14}H_{18}Cl_2NO_2^{1+}$	(126833-17-8)	M+nH	302.0709	10.05	1			1	1.4
Fenpyrazamine	F	$C_{17}H_{22}N_3O_2S^{1+}$	(473798-59-3)	M+nH	332.1427	9.76			2	2	2.7
Ferimzone	F	$C_{15}H_{19}N_4^{1+}$	(89269-64-7)	M+nH	255.1604	9.36		1		1	1.4
Fipronil (NH4)	I, VS	$C_{12}H_9Cl_2F_6N_5OS^{1+}$	(120068-37-3)	I	453.9725	10.5	18	2		20	27.0
Flufenacet	H	$C_{14}H_{14}F_4N_3O_2S^{1+}$	(142459-58-3)	M+nH	364.0737	10.06	18	16	18	52	70.3
Fluopicolide	F	$C_{14}H_9Cl_3F_3N_2O^{1+}$	(239110-15-7)	M+nH	382.9727	9.5			4	4	5.4
Fluopyram	F	$C_{16}H_{12}ClF_6N_2O^{1+}$	(658066-35-4)	M+nH	397.0537	9.86	20	13	9	42	56.8
Flurtamone	H	$C_{18}H_{15}F_3NO_2^{1+}$	(96525-23-4)	M+nH	334.1049	9.14	10	9	8	27	36.5
Fluxapyroxad	F	$C_{18}H_{13}F_5N_3O^{1+}$	(907204-31-3)	M+nH	382.0973	9.61	16	20	21	57	77.0
Griseofulvin	F, VS	$C_{17}H_{18}ClO_6^{1+}$		M+nH	353.0786	8.04	25	24	25	74	100.0

Imazalil	F, VS	$C_{14}H_{15}Cl_2N_2O^+$	(35554-44-0)	M+nH	297.0556	9.09	19			19	25.7
Imazamox	H	$C_{15}H_{20}N_3O_4^{1+}$	(114311-32-9)	M+nH	306.1448	4.63		1	1	2	2.7
Imidacloprid	I, VS	$C_9H_{11}ClN_5O_2^{1+}$	(138261-41-3)	M+nH	256.0596	4.76	25	24	25	74	100.0
Isopyrazam	F	$C_{20}H_{24}F_3N_2O^{1+}$	(881685-58-1)	M+nH	360.1882	11.56	3	4	4	11	14.9
Linuron	H	$C_9H_{11}Cl_2N_2O_2^+$	(330-55-2)	M+nH	249.0192	9.3	4	1	1	6	8.1
Maleic Hydrazide	H, PGR	$C_4H_5N_2O_2^{1+}$	(123-33-1)	M+nH	113.0346	1.7		1		1	1.4
Mepronil	F	$C_{17}H_{20}NO_2^{1+}$	(55814-41-0)	M+nH	270.1489	9.62	6			6	8.1
Mesosulfuron-methyl	H	$C_{17}H_{22}N_5O_9S_2^+$	(208465-21-8)	M+nH	504.0853	7.94	3	3	3	9	12.2
Metazachlor Fragm 210	H	$C_{11}H_{13}ClNO^{1+}$	(67129-08-2)	I	210.068	8.11	4	16	18	38	51.4
Metconazole	F	$C_{17}H_{23}ClN_3O^{1+}$	(125116-23-6)	M+nH	320.1524	11.21	3		1	4	5.4
Methomyl D3	I, Ac, Me	$C_5H_8D_3N_2O_2S^{1+}$	(1398109-07-3)	M+nH	166.0724	4.16	3			3	4.1
Methothrin	I	$C_{19}H_{27}O_3^{1+}$	(34388-29-9)	M+nH	303.1955	12.47	3	2	3	8	10.8
Metobromuron	H	$C_9H_{12}BrN_2O_2^+$	(3060-89-7)	M+nH	259.0077	8.15			3	3	4.1
Metolachlor	H	$C_{15}H_{23}ClNO_2^{1+}$	(51218-45-2)	M+nH	284.1412	10.29		6	5	11	14.9
Metrafenone	F	$C_{19}H_{22}BrO_5^{1+}$	(220899-03-6)	M+nH	411.0627	11.43			3	3	4.1
Metribuzin	H	$C_8H_{15}N_4OS^{1+}$	(21087-64-9)	M+nH	215.0961	7.07	3			3	4.1
Metsulfuron-methyl	H	$C_{14}H_{16}N_5O_6S^{1+}$	(74223-64-6)	M+nH	382.0816	6.31	1	2		3	4.1
Monolinuron	H	$C_9H_{12}ClN_2O_2^{1+}$	(1746-81-2)	M+nH	215.0582	7.76	1			1	1.4
Monuron	H	$C_9H_{12}ClN_2O^{1+}$	(150-68-5)	M+nH	199.0633	6.9	1			1	1.4
Myclobutanil	F	$C_{15}H_{18}ClN_4^{1+}$	(88671-89-0)	M+nH	289.1215	9.74	1			1	1.4
N.N-Dimethyl-N'-p-tolylsulphamide	Me	$C_9H_{15}N_2O_2S^{1+}$	(66840-71-9)	M+nH	215.0849	7.25	4			4	5.4
Naphthalene acetamide	PGR	$C_{12}H_{12}NO^{1+}$	(86-86-2)	M+nH	186.0913	6.56	1			1	1.4
Napropamide	H	$C_{17}H_{22}NO_2^{1+}$	(15299-99-7)	M+nH	272.1645	10.18		1	1	2	2.7
Nicotine	I, LC	$C_{10}H_{15}N_2^{1+}$		M+nH	163.123	2.41	5			5	6.8
Oxadiazon	H	$C_{15}H_{19}Cl_2N_2O_3^{1+}$	(19666-30-9)	M+nH	345.0767	12.35	11			11	14.9
Oxfendazole	I, VS	$C_{15}H_{14}N_3O_3S^{1+}$	(53716-50-0)	M+nH	316.075	6.62	11	2		13	17.6
Oxydemeton Methyl Sulfone	I	$C_6H_{15}O_5PS_2^{1+}$	(17040-19-6)	M+nH	263.0171	4.12		1		1	1.4
Penconazole	F	$C_{13}H_{16}Cl_2N_3^{1+}$	(66246-88-6)	M+nH	284.0716	10.83	1	1		2	2.7
Penthiopyrad	F	$C_{16}H_{21}F_3N_2OS^{1+}$	(183675-82-3)	M+nH	360.1352	10.72		5	9	14	18.9
Piperonylbutoxide Fragm 177	S	$C_{11}H_{13}O_2^{1+}$	(51-03-6)	I	177.091	12.37	8			8	10.8
Prometryn (Caparol)	H	$C_{10}H_{20}N_5S^{1+}$	(7287-19-6)	M+nH	242.1434	10.06	21	3	7	31	41.9
Propiconazole I	F	$C_{15}H_{18}Cl_2N_3O_2^{1+}$	(60207-90-1)	M+nH	342.0771	10.93	25	24	25	74	100.0
Propiconazole II	F	$C_{15}H_{18}Cl_2N_3O_2^{1+}$	(60207-90-1)	M+nH	342.0771	11.05	25	24	25	74	100.0
Propyzamide (Pronamide)	H	$C_{12}H_{12}Cl_2NO^{1+}$	(23950-58-5)	M+nH	256.029	9.69	24	23	25	72	97.3
Prosulfocarb	H	$C_{14}H_{22}NOS^{1+}$	(52888-80-9)	M+nH	252.1417	11.92	5			5	6.8
Prothioconazole desthio	Me	$C_{14}H_{16}Cl_2N_3O^+$	(120983-64-4)	M+nH	312.0665	10.43	17	21	24	62	83.8
Pyracarbolid	F	$C_{13}H_{16}NO_2^{1+}$	(24691-76-7)	M+nH	218.1176	7.24			1	1	1.4
Pyrethrins: Cinerin I	I	$C_{20}H_{29}O_3^{1+}$	(25402-06-6)	M+nH	317.2111	13	5	10	7	22	29.7
Pyrethrins: Cinerin II	I	$C_{21}H_{29}O_5^{1+}$	(121-20-0)	M+nH	361.201	11.55	5			5	6.8
Pyrethrins: Jasmolin I	I	$C_{21}H_{31}O_3^{1+}$	(4466-14-2)	M+nH	331.2268	13.35	6	1	1	8	10.8
Pyrimethanil	F	$C_{12}H_{14}N_3^{1+}$	(53112-28-0)	M+nH	200.1182	9.44			1	1	1.4
Pyroquilon	F	$C_{11}H_{12}NO^{1+}$	(57369-32-1)	M+nH	174.0913	6.9		1		1	1.4
Pyroxsulam	H	$C_{14}H_{14}F_3N_6O_5S^{1+}$	(422556-08-9)	M+nH	435.0693	6.72		1		1	1.4
Quinmerac	H	$C_{11}H_9ClNO_2^{1+}$	(90717-03-6)	M+nH	222.0316	4.75	12	10	12	34	45.9
Silthiofam	F	$C_{13}H_{22}NOSSi^+$	(175217-20-6)	M+nH	268.1186	10.58	3	6	4	13	17.6
Simazine	H	$C_7H_{13}ClN_5^{1+}$	(122-34-9)	M+nH	202.0854	7.07	2	2	2	6	8.1
Tebuconazole	F	$C_{16}H_{23}ClN_3O^{1+}$	(107534-96-3)	M+nH	308.1524	10.87	19	17	16	52	70.3
Tebutame	H	$C_{15}H_{24}NO^{1+}$	(35256-85-0)	M+nH	234.1852	10.25	1			1	1.4
Terbuthylazine	H	$C_9H_{17}ClN_5^{1+}$	(5915-41-3)	M+nH	230.1167	9.36	6	10	13	29	39.2
Terbutryn	H	$C_{10}H_{20}N_5S^{1+}$	(886-50-0)	M+nH	242.1434	10.23	22	5	13	40	54.1
Thiacloprid	I	$C_{10}H_{10}ClN_4S^{1+}$		M+nH	253.0309	5.62	1		2	3	4.1
Triphenylphosphate	Ad, FR	$C_{18}H_{16}O_4P^{1+}$	(115-86-6)	M+nH	327.0781	11.01	25	22	25	72	97.3
Uniconazole	F	$C_{15}H_{19}ClN_3O^{1+}$	(83657-22-1)	M+nH	292.1211	10.46	10	13	13	36	48.6
Warfarin	Ro	$C_{19}H_{17}O_4^{1+}$	(81-81-2)	M+nH	309.1121	9.27	16	5	2	23	31.1

**Peak 1 and Peak 2 refer to compounds with cis/trans isomers with closely eluting retention times and identical diagnostic mass ions. I and II refer to isomers which are indistinguishable by mass spectrometry. Where Frag follows the compound name identification is made with a fragment ion of mass given. ** Additive; Ad, Acaricide; Ac, Flame retardant; FR, Fungicide; F, Herbicide; H, Insecticide; I, Lifestyle compound; LC, Metabolite; Me, Molluscicide; Mo, Nematicide; N, Plant growth regulator; PGR, Repellent; Re, Rodenticide; Ro, Synergist; S, Veterinary substance; VS.*

Herbicides were the largest group with 37 detections, closely followed by fungicides with 36 detections. Twenty-two insecticides were detected along with ten pesticide metabolites. The remaining six detections were composed of rodenticides, repellents and compounds used in pesticide manufacturing or within pesticide formulations alongside an active compound (Fig S4). Many of the 111 detected analytes have non-pesticide applications, for instance warfarin is an anti-coagulant prescription medication which was historically used as a rodenticide. A number of detected analytes also have veterinary uses such as the neonicotinoid insecticide imidacloprid, which is widely used in flea treatments. Previous screening studies of spot samples taken from rivers within the European Union identified a similar number of compounds (approximately 100), including many of the compounds identified in this work.[30,31] The proportion of herbicides, fungicides and insecticides detected were likewise broadly similar to the current study. A recent study of groundwater using passive sampling identified 45 pesticides.[20] In these studies many of the most frequently detected compounds are shared (e.g. diuron).

Spatial Trends

The frequency of detection varied greatly for different analytes with some analytes ubiquitous at all sites throughout the study period. Table 1 details the number of detections at each site and the detection frequency in all 74 samples. Other analytes were ubiquitous at certain sites only. Five analytes were detected in 100% of samples. Twenty-four analytes were detected in at least 50% of samples. Twenty-eight analytes were detected in between 10-50% of samples. Most of the analytes were present infrequently with 59 analytes detected in fewer than 10% of samples, with approximately one third of these detected on only one occasion. Fig. S5 details the number of detections at each site for the 111 analytes.

Fewer analytes were detected at each site progressively through the catchment, with 86, 71 and 67 analytes detected at site 1, 2 and 3, respectively (Fig. 3). This decrease may seem counter intuitive given the increase in the size of the corresponding upstream catchment, however, instream attenuation through dilution and degradation processes may result in pesticide concentrations below method sensitivity at downstream locations. The relative dominance of specific sources of pollution is likewise expected to be greater at upstream sites. Poulier *et al*.[14] detected fewer pesticides in POCIS deployed at an upstream site than a site downstream, observing an increase in concentration with progress downstream for compounds present at both sites. This result was attributed to the relative importance of diffuse inputs between the two sites. Aguilar *et al*.[32] observed a greater number of pesticides in spot samples at downstream sites in a river catchment dominated by agricultural inputs. The importance of inputs above site 1 may explain the reduction in the number of analytes present with increases in catchment size observed in this work, as inputs originating from point sources

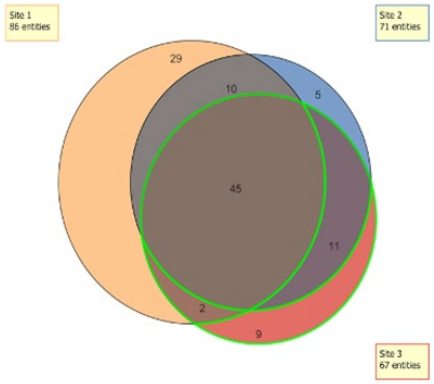

Fig. 3 Venn diagram showing the number of analytes detected at each site and the number of analytes detected at multiple sites. Highlighted entities represent analytes present at site 3.

(i.e. WWTW) are not supported by multiple inputs throughout the catchment. Site 1 had the greatest number of unique analytes (27), followed by site 3 (9), and site 2 (5). Ten analytes detected at site 1 were also present at site 2, but absent downstream. Eleven analytes were present at site 2 and site 3 indicating sources downstream of site 1. Two analytes were present at site 1 and site 3 indicating that site 1 is not the source of this pollution at site 3. Analytes present at all sites may result from inputs originating throughout the catchment, or from inputs upstream of site 1. The 67 analytes detected at site 3 can be split into 4 groups (highlighted in Fig. 3) based on Venn position to rationalise possible catchment sources of pollution at the WSW abstraction (site 3).

Temporal Trends

Unsupervised HCA was applied to normalised data (Z-transformed) for mean peak abundance for each deployment to reduce the complexity of the large data set. Clustering on entities (analytes) and conditions (deployments) was performed so that temporal trends shared by groups of entities could be visualised. Entities were separated by a cluster distance of 20 (Euclidean distance metric). Reducing the cluster distance to approximately eight produced six clusters of entities with similar temporal trends, within which two clusters were separated by a distance of approximately 11. Deployments were separated by a greater cluster distance (22). Reducing the cluster distance to approximately 15 grouped deployments into two clusters. Cluster A contained deployments 15-25 whilst cluster B contained deployments 1-14. Cluster A corresponds to summer and early autumn, whilst cluster B corresponds to late autumn, winter and spring. Deployment 1-4 occurred in autumn 2017 whereas deployments 23-25 occurred in autumn 2018. Clusters 1-6 contained 18, 16, 27, 14, 9 and 27 analytes respectively.

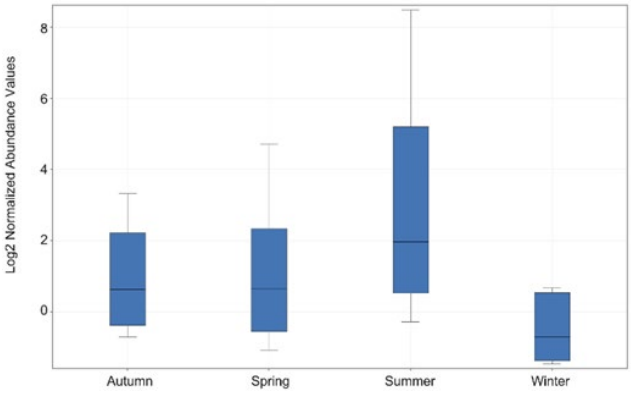

Fig.4 Box and whisker diagram of the average seasonal abundance of cluster 3 analytes at site 3.

Fig. 4 shows cluster 3 analytes are associated with increased abundance in summer and reduced abundance in winter. The median and interquartile range (IQR) of autumn and spring (seasons separating the peak and trough) are similar differing only in the range. Detailed summaries of each cluster are presented in ESI Fig. S6. To investigate temporal trends between similar analytes in each cluster, box and whisker diagrams of the seasonal average normalised abundance of detected analytes at site 3 are presented alongside the detailed cluster summaries. Seasonal associations are observed for all clusters.

Characterisation of pesticide pollution

An example of characterisation of pesticide pollution was performed for cluster 3 analytes (Fig. 5). Site 3 was used as it was the location for a potable water abstraction and the significance of pesticide

pollution is greater than at upstream locations as a result. Data describing the properties of each pesticide has been taken from the Pesticide Properties Database, which is presented in Table S3.[33] Table 2 simplifies this information so that properties of cluster 3 analytes are accounted in a standardised way. This characterisation can be repeated for each cluster separately but is only performed for cluster 3 analytes here for brevity.

Nine analytes have current approval in the EU. Including the insecticide imidacloprid which is also approved for veterinary use. Two analytes are transformation products of approved pesticides present in the cluster; namely, diuron metabolites DCPMU and DCPU. Tetrahydrophthalimide is a metabolite of the approved fungicide captan. Carbofuran-3-hydroxy can be a metabolite of either carbofuran or benfuracarb, neither of which is currently approved. All four of these transformation products are formed in soil. Diuron is persistent in soil and its presence alongside its metabolites may suggest historic use.

Furthermore, local restrictions ban diuron use in the United Kingdom supporting a legacy origin of this pollutant. Fourteen analytes have no current approval in the EU, including compounds without past approval. Of the nine analytes approved for use in the EU only fungicides dimethomorph and penconazole, the insecticides pyrethrins: Cinerin II, imidacloprid and thiacloprid, and the plant growth regulator, 1-naphthylacetic acid had local approval at the time of sampling.

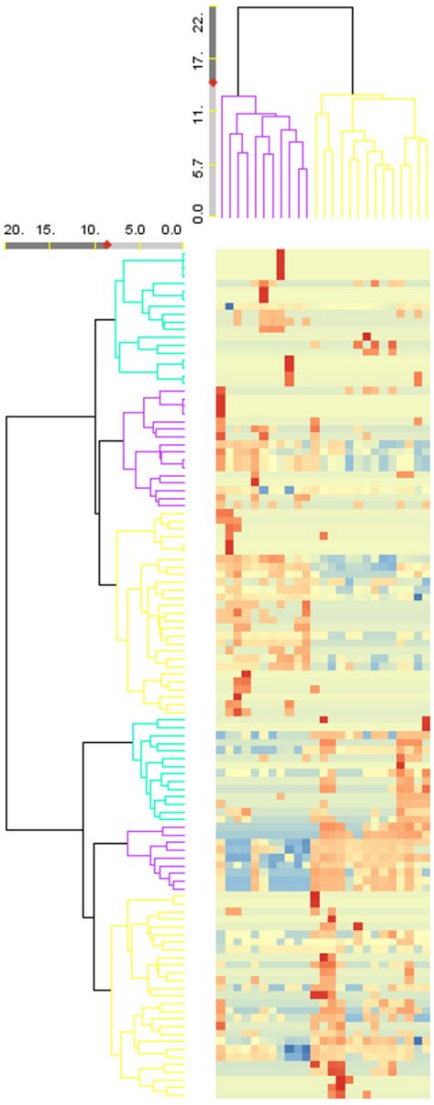

Fig. 5 Hierarchical clustering of normalized intensity values for entities (clusters 1–6) and conditions (clusters A and B). Conditions represent an average value for each of Deployment 1–25. Similarity Measure: Euclidean. Linkage Rule: Wards. Tree scale: True.

Table 2 Accounting of the properties of pesticides in cluster 3.

	Approval EU				Crops			Setting			
	Current	None	TP	Biocide	Veg & root	Fruit	Cereal	Agriculture	Veterinary	Urban	Count
Herbicides	**2**	**2**	**2**	**0**	**2**	**2**	**1**	**4**	**0**	**1**	**16**
DCPMU (Monomethyldiuron)			x								
DCPU (1.3.4-dichlorophenyl-urea)			x								
Diuron	x				x	x	X	x		x	
Metobromuron	x				x	x		x			
Prometryn (Caparol)		x						x			
Terbutryn		x						x			
Fungicides	**3**	**3**	**1**	**0**	**5**	**4**	**1**	**6**	**0**	**2**	**25**
1.2.3.6-Tetrahydrophthalimide (cis-)			x								
Dimethomorph Peak 1	x				x	x		x			
Dimethomorph Peak 2	x				x	x		x			
Fenamidone		x			x	x		x			
Penconazole	x				x	x		x		x	
Pyracarbolid		x			x			x		x	
Pyroquilon		x					X	x			
Insecticides	**3**	**7**	**1**	**6**	**3**	**3**	**1**	**5**	**5**	**7**	**43**
Aldicarb-sulfone (Aldoxycarb) Fragm 148		x		x	x			x		x	
Carbofuran-3-hydroxy			x								
DEET (Diethyltoluamide)		x		x					x	x	
Diazinon		x		x		x		x	x	x	
Dioxacarb		x			x			X		x	
Fipronil (NH4)		x		x					x	x	
Imidacloprid	x			x	x		X	x	x	x	
Methothrin		x									
Oxfendazole		x		x					x		
Pyrethrins: Cinerin II	x					x				x	
Thiacloprid	x					x		x			
Other	**1**	**2**	**0**	**1**	**1**	**1**	**0**	**1**	**0**	**1**	**8**
1-Naphthylaceticacid (NH4)	x				x	x		x			
Piperonylbutoxide Fragm 177		x									
Warfarin		x		x						x	
Total	**9**	**14**	**4**	**7**	**11**	**10**	**3**	**16**	**5**	**11**	

Five these were approved for use on fruit suggesting a possible origin for these compounds. Seven analytes are used as biocides including the insect repellent DEET and the insecticide imidacloprid which are used in consumer products, suggesting a wastewater source for these compounds. Likewise, warfarin is an anticoagulant prescription medication with a probable wastewater source.

Fourteen analytes contained in cluster 3 were detected at site 3. Fig. S7 presents the seasonality of these detections. Five analytes were detected in all seasons. None of these have current approval. All 14 analytes were detected in summer. The most likely source for cluster 3 analytes is continuous inputs from legacy pollution originating from groundwater, and/or ongoing discharges in wastewater. The abundance of these compounds decreases with progress through the catchment suggesting a catchment source upstream of site 1. Any future monitoring programme for cluster 3 analytes should focus on site 1 and site 3, with an expanded suite in summer. Catchment interventions to reduce pollution for current use pesticides should focus on potential point sources, such as run-off from greenhouses draining to the sewage system.

Risk assessment of pesticides at water supply works abstraction

The 67 analytes detected at site 3 were considered in the risk assessment. An initial risk score was assigned to each analyte based on detection frequency at site 3. These scores are high (> 20), medium (10-19), low (5-9) and vlow (< 5). The position of analytes in the Venn diagram in (Fig. 3) was considered alongside the risk score and the seasonal trends of analytes in each cluster identified in the HCA (Fig. 5) presented in Fig. S6. Table 3 below presents a summary of the risk score for analytes in clusters 1-6 and the number of analytes prioritised for future monitoring, presented in detail in Table S4.

Table 3 Risk assessment and prioritisation of monitoring summary.

Risk Score	C1	C2	C3	C4	C5	C6	Sum
High	1	4	4		2	4	15
Medium		1	1	3	6	3	14
Low		1	1	3	1	3	9
vLow	6	3	8	3		9	29
Total	7	9	14	9	9	19	67

Prioritised*	Site 1	Site 2	Site3
Autumn	17	16	38
Winter	26	28	41
Spring	35	35	42
Summer	12	16	32
Total	45	54	67

*no. analytes prioritised for future monitoring.

Fifteen pesticides were assigned a high score and prioritised for monitoring throughout the year. Trends in the seasonality and spatial variability of analytes with a lower score informed the prioritisation of monitoring by site and season. The prioritised monitoring list condensed the monitoring suite to between 32 and 42 analytes per season, with a further reduction in the suite at upstream sites.

Typically risk assessments are based on risk or hazard quotients relating the measured concentration of individual pesticides in discrete samples to a threshold concentration. Such approaches neglect the complexity of the processes contributing to pesticide fate in surface waters and highly fluctuating concentrations that result. As such the timing of sampling can significantly influence any risk assessment informed by discrete sampling methods. The approach used in this work cannot distinguish between episodic or regular pesticide pollution but captures both through time integrative sampling. As such this approach is unlikely to omit pesticides of high consequence within the

catchment from the risk assessment or misinterpret risk due to intermittent presence or variable concentrations. However, only tentative conclusions may be drawn in the absence of quantitative data on pesticide concentration. This work adds to the number of studies using passive samplers to assess PPPs sources, pathways and fate at the catchment level. [14,19,34-38]

Conclusions

The presented method characterises pesticide presence and fate in a river catchment and assesses the risk from pesticide pollution at a water supply works abstraction. This was undertaken with the objective of improving water quality management, through improved monitoring and data handling. To this end we coupled several commercially available technologies in a novel way to provide new insight without the need to expand the scope of monitoring or incur additional costs. The standardised method combining passive sampling, targeted screening and multi-variate statistics can characterise spatial and temporal trends, and discriminate similarities within and between groups of analytes from a large qualitative dataset. One hundred and eleven pesticides were detected in the catchment over 12 months. A seasonally prioritised monitoring programme reduced the monitoring suite significantly at each site based on risk. In future a combination of passive sampling and chemometrics may prove a useful tool for directing quantitative analysis and designing monitoring programmes. Used in isolation such qualitative assessment of pesticide pollution can only characterise risk. However, this method addresses data gaps arising from infrequent sampling and/or relevant analytes omitted in routine analytical suites and is complimentary to monitoring and analytical methods employing spot sampling and quantitative analysis. This approach may also have applications in mixture toxicity assessment, where efforts to relate measured environmental concentrations to ecological threshold environmental quality standards and predicted-no-effect concentrations are frustrated by the availability and quality of data for mixtures

Conflicts of interest

There are no conflicts to declare.

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