

Fluorescence spectroscopy as an indicator tool for pharmaceutical contamination in groundwater and surface water

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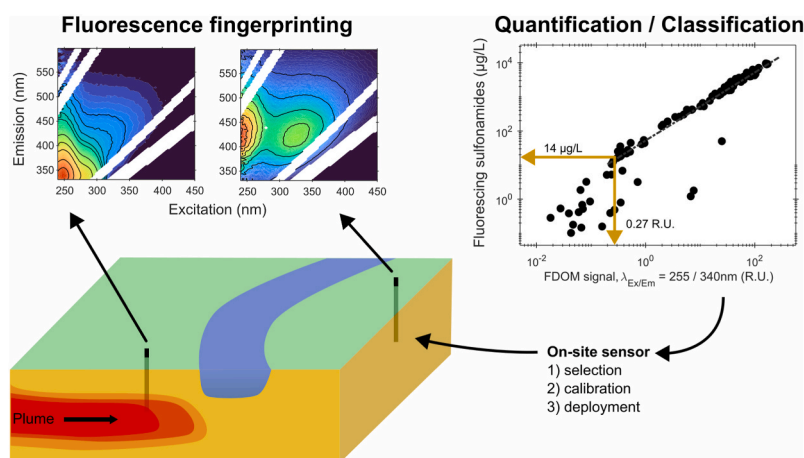
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HIGHLIGHTS

- Three sulfonamides fluoresce strongly with high spectral similarity.
- Fluorescence is a viable indicator to track sulfonamide plumes in groundwater.
- Screening during field investigations with sensor at threshold concentration of 142 µg/L.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling Editor: Myrto Petreas

Keywords:

Fluorescence
Sulfonamides
Pharmaceutical compounds
Groundwater contamination

ABSTRACT

Knowledge of contaminant distribution and transport of contaminant plumes in groundwater is important for effective remediation. Tedious and expensive laboratory analyses could be supplemented with optical measurements such as fluorescence to offer a rapid alternative with the potential for on-site measurements. Here, we explore the applicability of fluorescence spectroscopy as an on-site alternative to identifying the extent of a groundwater contaminant plume in Grindsted, Denmark. We show that three abundant contaminants (sulfanilamide, sulfaguanidine, and sulfanilic acid) emit very strong, but highly similar fluorescence distinct from the naturally occurring organic matter. The limit of detection for the sum of these three contaminants was 14 and

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<https://doi.org/10.1016/j.chemosphere.2024.144009>

Received 29 October 2024; Received in revised form 19 December 2024; Accepted 20 December 2024

Available online 18 January 2025

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Field investigations
Screening tool

142 µg/L using benchtop measurements and handheld sensors, respectively. We demonstrate that low-volume solid-phase extractions can be a tool to lower the detection limits through the selective enrichment of contaminants. However, the co-occurrence of natural and anthropogenic fluorescent organic matter presents a significant challenge for the reliable quantification of contaminants. The high similarity between investigated fluorescent contaminants poses a significant challenge for machine learning approaches that are commonly used to increase sensitivity and selectivity. Nonetheless, the results demonstrate how fluorescence spectroscopy can be applied as a viable indicator and classification tool to identify pharmaceutical contamination in groundwater, as well as surface waters.

1. Introduction

Awareness of anthropogenic contaminants in the environment continues to grow significantly due to an increased number of studies investigating groundwater and surface water bodies (Lapworth et al., 2012). Increased focus has, amongst others, been on pharmaceutically active compounds (Lapworth et al., 2012; D'Alessio et al., 2016; Halting-Sørensen et al., 1998; Daughton, 2016; Kümmerer, 2009). Pharmaceutical contamination in the environment can originate from sources such as wastewater treatment plants, septic tanks, sewage systems, and manure from livestock production (Lapworth et al., 2012; Bottoni et al., 2010). Fewer studies have focused on pharmaceuticals originating from contaminated sites such as former manufacturing sites and uncontrolled landfills, though these constitute a risk to water quality in the surrounding area due to especially high contaminant loads impacting the soil and groundwater (Balbarini et al., 2020; Bennett et al., 2017; Larsson, 2014). Contaminant plumes from these sources can spread significantly in groundwater, and result in the contamination of surface water bodies due to interactions between groundwater and surface water (D'Alessio et al., 2016; Holm et al., 1995; Balbarini et al., 2018). As the presence of pharmaceuticals in the environment has only gained interest to society recently, little is yet known about the environmental fate and ecological impacts of many of these compounds (Bottoni et al., 2010). Experimental insights indicate potential negative effects of various pharmaceuticals on human health and ecosystem functioning (Bottoni et al., 2010). Another concerning aspect of pharmaceutical contamination is the risk of antibacterial resistant strains that delay and limit treatment options (Kergoat et al., 2021; Deng et al., 2018; Hruska and Franek, 2012; Sarmah et al., 2006). These negative effects highlight the need to better understand the fate and transport of pharmaceutical contaminant plumes.

This study focusses on a known contamination plume of a former pharmaceutical manufacturing facility in Grindsted, Denmark. The plume contains various groups of pharmaceuticals (including antibiotics and sleep-inducing drugs), as well as other compounds related to pharmaceutical production, such as benzene and chlorinated ethenes (Balbarini et al., 2018; Sonne et al., 2017; Ottosen et al., 2020; Rønde et al., 2017). Rapid screening for benzene and chlorinated ethenes is possible using e.g. photoionization detection or halogen sensitive detection via a membrane interface probe (MIP) measuring the volatile hydrocarbon contamination on site (Adamson et al., 2014; McCall et al., 2014). Contrary to this, the standard procedure for pharmaceutical contamination investigations relies on liquid chromatography-mass spectrometry (LC-MS) in certified laboratories to quantify the abundances of specific compounds. Consequently, the costs for quantitative analyses are high. As a result, field scale mapping of the extent of pharmaceutical contamination is both time consuming and very costly, and has likely acted as a barrier to the in-depth investigation of these chemicals. Therefore, it would be beneficial to develop a low-cost and fast screening tool for pharmaceutical compounds in the subsurface environment.

In recent years, fluorescence spectroscopy has been widely implemented as an analytical tool for the characterization of natural dissolved organic matter (DOM) in biological and environmental science (Lakowicz, 2006; Coble et al., 1998). The technique targets the

fluorescent fraction of dissolved organic matter (FDOM) and represents a rapid and low-cost alternative to advanced instrumentation such as mass spectrometry. Studies often use three-dimensional Excitation-Emission Matrices (EEMs) to fingerprint different samples, as well as to investigate the link between DOM composition and e.g. the mobility of trace metals such as arsenic (Schittich et al., 2018; Li et al., 2016). EEMs have also been used as a surrogate technique to track fluorescent pharmaceuticals, study their degradation processes, and monitor treatment efficiency over time in wastewater treatment plants (Roccaro et al., 2020; Sgroi et al., 2017; Ouarda et al., 2019). Since fluorescent compounds (fluorophores) are typically aromatic, many pharmaceuticals can theoretically be detected. However, a calibration must be carried out for each specific instrument and/or study to relate fluorescence observations to concentrations (Lakowicz, 2006).

For investigating freshwater systems or groundwater sites with low contaminant concentrations, it can be desirable to improve contaminant detectability prior to fluorescence analysis. In such cases, selective enrichment of the contaminants is required to isolate their fluorescence from the natural background. This selective enrichment makes it more likely that the targeted fluorescence spectra emerge over the background and thus improves classification performance. A versatile and highly popular approach to achieving the extraction of organic compounds from aquatic samples and their enrichment is solid-phase extraction (Dittmar et al., 2008). Studies have shown that different sorbents are well-suited for extractions of specific subfractions of DOM and are less efficient for other subfractions, thus allowing to separate different DOM fractions (Dittmar et al., 2008; Rosario-Ortiz et al., 2004). While the acidification of samples maximizes the extraction efficiency, the isolation of material under ambient conditions can also be used to selectively isolate subfractions of DOM (Rosario-Ortiz et al., 2007; Wünsch and Murphy, 2021).

The overall goal of this study was to develop a fluorescence-based screening tool for pharmaceutical contamination in groundwater and surface water. The study aimed to (1) identify relevant pharmaceutical compounds/compound groups capable of emitting fluorescence and establish their fluorescence properties, (2) evaluate whether fluorescing contaminants can be distinguished from the natural background fluorescence, (3) determine whether the fluorescing contaminants have detection limits suitable for on-site screening efforts during field investigations and (4) investigate whether solid-phase extraction (SPE) at circumneutral pH and with small sample volumes is suitable to lower the detection limit of relevant fluorescent contaminants.

2. Materials and methods

2.1. Study site description

The study site is located in the town of Grindsted in the Region of Southern Denmark, where a former pharmaceutical production facility has resulted in a large contaminant plume discharging to Grindsted stream. The complex groundwater contaminant plume is composed of various compound groups; pharmaceuticals, benzene, and chlorinated ethenes (Balbarini et al., 2020; Sonne et al., 2017; Rønde et al., 2017). The pharmaceuticals include sulfonamides and barbiturates, as well as sulfanilic acid, which was used in the production of sulfa drugs and is

also a potential degradation product of these (Hu et al., 2022; Reis et al., 2020). In the plume, the contaminants are found at very high concentrations ($\mu\text{g/L}$ to mg/L scale). A study conducted in 2020 investigated the development of the contaminant plume and suggested that the majority of the source at the factory site was depleted and that the core of the plume was closer to the stream (Balbarini et al., 2020). Sampling from 2015 to 2016 revealed chlorinated ethene concentrations up to $1700 \mu\text{g/L}$ and sulfanilamide concentrations up to $880 \mu\text{g/L}$ in a monitoring well midway between the factory site and the stream (Balbarini et al., 2020). Furthermore, numerous samples with sulfonamide concentrations $>100 \mu\text{g/L}$ were found close to the stream in the period 2012–2016 (Balbarini et al., 2020). The pharmaceutical contamination is known to discharge to the stream along a 2 km stretch and can be detected as far as 16 km from where the plume enters the stream (Balbarini et al., 2018; Sonne et al., 2017; Rasmussen et al., 2016). In order to initiate remediation at the site, it is crucial to have an in-depth understanding of the discharge of contaminants to the stream and hence extensive investigations of the contaminant plume close to the stream are necessary.

2.2. Investigation strategy and sampling

In earlier investigations, groundwater samples for fluorescence and absorbance measurements were collected at the site. These showed very intense fluorescence spectra, which could not be explained by the presence of natural dissolved natural organic matter (NOM) in the samples. Protein-like fluorescence signals were extremely high and spectrally not congruent with amino-acid fluorescence spectra commonly seen in typical ground- or stream water NOM (Wünsch et al., 2019). This suggested that the observed fluorescence in the groundwater samples came from specific compounds or compound groups present in the contaminant plume, and that this plume could therefore be monitored with spectroscopic instruments.

The spectral properties of selected compounds representing all compound groups present in the contaminant plume at the study site were determined. Initially, nine compounds targeting seven different compound groups were analyzed. As strong fluorescence was observed for some sulfonamides, including sulfanilamide, it was decided to further investigate eight compounds within this compound group. This results in a total of 17 screening compounds (2 chlorinated ethenes, 1 barbiturate, 9 sulfonamides, sulfanilic acid, benzene, naphthalene, aniline and nikethamide) for which fluorescence and absorbance properties were determined (as described below). A list of all analyzed

compounds along with the observed spectral properties is provided in Table 1.

One hundred and forty groundwater grab samples were collected for chemical analysis (targeting sulfonamides) as well as fluorescence and absorbance measurements from drive point sampling points close to the stream bank in the period of August–November 2021 at depths between 1 and 20 m below ground level. Samples sent for fluorescence and absorbance analysis in the laboratory were filtered through a $0.45 \mu\text{m}$ cellulose acetate filter (Minisart NML, Sartorius Stedim Biotech GmbH) to remove particulate matter. The first 5 mL of each filtrate were discarded to avoid biases caused by adsorption to or leaching of filter material. Hydrophilic cellulose acetate was specifically chosen to minimize sorption of analytes. All samples were stored at 4°C in the dark (until analyzed within 2 weeks of sampling). Storage effects were assumed to be negligible since intervals between sampling and measurements were kept as similar as logistically feasible. Freezing was disregarded as a storage solution to avoid significant impacts of freeze/thaw cycles on fluorescence properties (Spencer et al., 2007). For 80 of the 140 samples, fluorescence was screened with a sensor on site (further information below).

2.3. Targeted chemical analysis

Sulfonamides concentrations in the 140 samples were determined after filtration (no solid-phase extraction) by High Performance liquid Chromatography-Triple Quadrupole Mass Spectrometry. Deuterium labelled standards of the compounds were used as internal standards and the detection limits were $0.2\text{--}0.5 \mu\text{g/L}$. Chromatographic separation was achieved on a $2.1 \times 100 \text{ mm}$, $3 \mu\text{m}$, $120 \text{ \AA}$ reversed phase column with 0.1% formic acid as aqueous phase (A) and acetonitrile with 0.1% formic acid as mobile phase (B). Detection was achieved by dynamic Multiple Reaction Monitoring (dMRM). All compounds were measured using positive mode electrospray ionization (ESI+) except sulfanilic acid, which was detected using ESI in negative mode.

2.4. Spectroscopic measurements

All samples for fluorescence and absorbance measurements were filtered before analyses in a 10 mm quartz cuvette by a HORIBA Aqua-Log fluorometer. Before sample measurements, a daily calibration for the excitation monochromator and the emission detector was conducted, as well as a validation of optical immaculacy of measurement cuvettes following a previously described protocol (Wünsch et al., 2015).

Table 1

Summary of optical properties for relevant pure compounds dissolved in pure water at pH 7. “-” refers to compounds that did not fluoresce at 1 mg/L , “N.F.” = no fluorescence, “N.D.” = not detectable. The peak wavelengths are determined with an accuracy of $\pm 1 \text{ nm}$ as per manufacturer specifications. For compound spectra, see Fig. S2.

Compound	CAS no.	$\lambda_{\text{Em, max}}$ (nm)	$\lambda_{\text{Ex, max}}$ (nm)	Fluorescence per mg (R.U. $\text{mg}^{-1} \text{ L}$)	Absorbance per mg ($\text{mg}^{-1} \text{ L}$ cm^{-1})	Fluorescence at median study concentration (R.U.)
Sulfanilic acid	121-57-3	337	248	8.8	0.04	15.6
Sulfaguanidine	57-67-0	340	258	7.7	0.15	0.09
Sulfanilamide	63-74-1	338	259	18.3	0.35	0.16
Sulfacetamide	144-80-9	337	256	6.4	0.34	N.D.
Sulfamethazine	57-68-1	340	261	0.1	0.10	<0.01
Sulfadiazine	68-35-9	N.F.				
Sulfamethizole	144-82-0	N.F.				
Sulfamerazine	127-79-7	N.F.				
Sulfapyridine	144-83-2	N.F.				
Sulfathiazole	144-74-1	N.F.				
Vinyl chloride	75-01-4	N.F.				
cis-1,2-dichlorethen	156-59-2	N.F.				
Isopropylbarbituric acid	7391-69-7	N.F.				
Benzene	71-43-2	N.F.				
Naphthalene	91-20-3	322	274	33.8	0.04	N.D.
Aniline	62-53-3	337	280	10.4	0.06	N.D.
Nikethamide	59-26-7	N.F.				

Absorbance was determined between 240 and 600 nm and fluorescence EEMs were collected from 240 to 600 nm (excitation) and 200–620 nm (emission). Integration times were selected to adjust for varying levels of fluorescence in the measured samples. The data shown here have been restricted to fluorescence emission above 330 nm to focus on the spectral region relevant to the contaminants. Moreover, this choice was made because fluorescence data overlaps with Raman and Rayleigh scatter at short emission and excitation wavelengths and the quality of observation usually deteriorates.

Fluorescence and absorbance data were processed in MATLAB (v9.11, Mathworks Inc.) using the drEEM toolbox (v0.6.3) (Murphy et al., 2013). Using the absorbance-based approach, the inner filter effects were eliminated and fluorescence intensities were normalized using the Raman peak area at 350 nm (Kothawala et al., 2013). Furthermore, 1st and 2nd order physical scatter (Raman and Rayleigh scatter) was removed without interpolation. The slope of concentration vs fluorescence signal was used to determine molar fluorescence for pure compounds. To address the potential issue of selectivity, the spectral similarity between sample emission and the most abundant and fluorescent contaminant (sulfanilic acid) was quantified. For this purpose, Tucker's congruence coefficient (TCC) was used at $\lambda_{Ex} = 255$ and λ_{Em} between 330 and 379 nm (covering the emission peak of sulfanilic acid) (Lorenzo-Seva and ten Berge, 2006). Any similarity between 0.95 and 1 was deemed sufficient to conclude that the observed fluorescence was selective for the measured contaminants. Finally, the limit of detection (LOD) was calculated in accordance with the EPA method detection limit procedure (rev. 2, EPA document EPA 821-R-16-006) with the help of the instrument responses of samples showing contaminants below detection limit ("uncontaminated" as determined by the LC-MS method) and fluorescence as follows:

$$LOD = \bar{X} + t_{(n-1, 1-\alpha=0.99)} * S_b * m \quad (\text{Eq. 1})$$

$\bar{X}$ was the mean fluorescence of uncontaminated samples, t was the student's t -value, S_b was the standard deviation of the fluorescence of uncontaminated samples, and m was the slope of the linear regression between concentration and fluorescence. To derive m , samples with no detectable contamination of sulfanilic acid, sulfanilamide, and sulfaguanidine were excluded. The corresponding fluorescence cutoff was derived with the same methodology except for the multiplication with m . We acknowledge that there are several approaches for the determination of LOD with advantages and disadvantages.

2.5. On-site fluorescence sensor

An initial laboratory test of two different Turner fluorescence sensors, the Cyclops-7F Tryptophan Optics sensor and the Cyclops-7F Refined Fuels Optics sensor, was conducted on samples containing known concentrations of sulfanilamide and sulfanilic acid (1 µg/L, 10 µg/L, 100 µg/L and 1000 µg/L in demineralized water) before sensor deployment in the field. The Refined Fuels Optics sensor and the Tryptophan Optics sensor measure fluorescence at $\lambda_{Ex}/\lambda_{Em}$ 255/350, and 275/350, respectively. An initial test was carried out to identify which of the two sensors was better suited for the detection of sulfanilamide (a representative contaminant for the study). Based on the initial laboratory test, the Cyclops-7F Refined Fuels Optics sensor was selected for further investigations on field samples as it was approx. 10 times more responsive to pure sulfanilamide.

In the on-site setup, the Fuel Optics sensor was equipped with a flow through cap. The sensor data was logged through the manufacturer's software (Cyclops Explorer). The Fuel Optics sensor can collect data in three different gain settings (1x, 10x, 100x). The sensor system was calibrated using sulfanilamide and sulfanilic acid standards. When doing measurements, a sample was injected into the flow cap with a 20 mL sample syringe and fluorescence was measured using the most appropriate 10x gain setting. Before entering the flow cap the sample water

was filtered through a 0.2 µm filter to remove particulate matter. Measurements were collected over a 60 second period for each sample (or until a stable signal was observed). The median value over the 30–60s was noted down for subsequent data analysis. Since the flow-through cap gave a baseline sensor value due to scattering, a voltage of 180 mV was subtracted from the raw values for the analysis shown here. For the consideration of inner filter effects (IFE), the correction factor (IFC) was calculated using the data of the corresponding benchtop fluorometer sample using the absorbance-based approach as follows (Parker and Rees, 1960):

$$IFC = 10^{0.5 * (A_{255 \text{ nm}} + A_{350 \text{ nm}})} \quad (\text{Eq. 2})$$

2.6. Solid-phase extractions

Solid-phase extractions (SPEs) were carried out to extract contaminants from their matrix using the PPL sorbent (styrene-divinylbenzene reverse-phase sorbent, 200 mg, Agilent Technologies). While other sorbents are commercially available, we tested PPL as it is a common sorbent used across a wide range of studies (Dittmar et al., 2008). Extractions were carried out similar to the protocols described elsewhere (Wünsch and Murphy, 2021). A deviation from the established protocol was made by extracting at circumneutral pH to simplify the extraction protocol with potential field sampling campaigns in mind. To minimize time and effort and since the focus of this experiment was qualitative and not quantitative characterizations, extracts were subsequently characterized in methanol directly. Approximately 100 mL of the water sample was applied onto the SPE cartridge and extracts were eluted with 3 mL methanol. The extract fluorescence was then characterized in methanol as described above. Stream water samples for solid-phase extractions were collected upstream from the known contamination discharge areas. These were filtered through a 0.45 µm filter to remove particulate matter and stored in the dark at 4 °C (until analyzed).

3. Results and discussion

The most abundant fluorescent contaminants quantified in the contaminant plume, were sulfanilic acid, sulfaguanidine, and sulfanilamide, with median concentrations of 1700 µg/L, 10.9 µg/L, and 10.6 µg/L, respectively. The levels of all sulfonamide contaminants quantified in our study are listed in SI Figure S1. Though sulfanilic acid does not have active pharmaceutical properties itself (it is used in the production of the remaining sulfonamides), it is henceforth included as a sulfonamide for simplification. The dominant compound, sulfanilic acid accounted for approx. 90 % of sulfonamides on average. These concentrations agreed with previous studies of the Grindstedt site (Balbarini et al., 2018, 2020; Sonne et al., 2017; Rasmussen et al., 2016). To investigate how the contaminant plume could be tracked via the fluorescence of one or more of the most abundant contaminants, a screening of their optical properties was required.

3.1. Optical properties of pharmaceuticals

Strong fluorescence signals were observed for several compounds occurring at relevant concentrations at the study site; these mostly include sulfonamides (Table 1, Fig. S2).

No fluorescence was observed for the chlorinated ethenes, nikethamide, and the barbiturate compound, whereas some but not all aromatic compounds were fluorescent. These findings point to a natural limitation of fluorescence-based methods: mapping the transport and distribution of non-fluorescent compounds requires more time-consuming and expensive LC-MS based quantification methods and the potential decoupling between individual contaminants must be carefully considered in each case.

Naphthalene emitted the strongest fluorescence signal with a molar fluorescence of 33.8 R.U. x mg⁻¹ x L, whereas sulfanilamide emitted the

strongest fluorescence amongst the sulfonamides with a molar fluorescence of 18.3 R.U. $\times \text{mg}^{-1} \times \text{L}$. Except for aniline, all considered compounds showed only one major absorbance band. Though aniline and naphthalene emit strong fluorescence they have rarely been detected in the contaminant plume and never in very high concentrations and are therefore considered not detectable (N.D.) at the study site. At the median concentrations found in our study, the three most detectable compounds are sulfanilic acid (15.6 R.U.), sulfanilamide (0.16 R.U.) and sulfaguanidine (0.15 R.U.). In the following, we will refer to the sum of the concentrations of these three compounds as *sum of three fluorescent sulfonamides*, or $\sum_{\text{FSA}}$. While sulfanilic acid was by far the dominant compound, spatial variations in the plume composition might introduce a significant disturbance in the correlation between $\sum_{\text{FSA}}$ and fluorescence due to the variation in molar fluorescence amongst the three compounds.

An analysis of spectral similarity was conducted to identify whether individual fluorescent compounds could be distinguished with the help of machine learning approaches such as PARAFAC (Fig. S3). While excitation spectra were more distinct, there was a significant degree of spectral similarity between the emission spectra of many compounds. For example, the most prominent fluorophore amongst the samples investigated here (sulfanilic acid) had a well distinguished excitation spectra compared to the next two prominent fluorophores (sulfanilamide and sulfaguanidine, TCC = 0.87). However, the emission spectra were highly similar with TCCs of 0.99 and 0.98, respectively. At such high similarities, the variability assumption behind the PARAFAC model would likely be violated and PARAFAC could not reliably distinguish between individual fluorescent contaminants at this study site. The high degree of spectral similarity presents a significant challenge in terms of specificity. Individual contributions of spectrally indistinguishable contaminants cannot be quantified with optical measurements alone. We address this challenge by referring to the sum of fluorescent sulfonamides ($\sum_{\text{FSA}}$).

3.2. Fluorescence composition of field samples

The maximum fluorescence signal varied by several orders of magnitude between the different groundwater samples. Samples could essentially be classified into two subgroups. In the first group, natural humic-like fluorescence at emission wavelengths 400–500 nm were dominant in the EEMs (Fig. 1A) and

natural protein-like fluorescence at emission wavelengths 300–380 nm were low (<0.5 R.U.), resulting in a humification index (HIX_{Ohno}) of >0.5 (Ohno, 2002). Such samples will henceforth be referred to as uncontaminated samples. It should be noted that these samples might contain non-fluorescent contaminants, since our classification is purely based on fluorescence observations. The fluorescence intensity of non-contaminated samples varied between 0 and 0.88 Raman Units (R.U.), presumably originating from natural fluorescence. These intensities are in agreement with environmental samples from other studies (Fellman et al., 2010). Across all samples, the fluorescence of peak D (Lochmüller and Saavedra, 1986; Stedmon et al., 2003; Murphy et al., 2018; Ishii and Boyer, 2012) ($\lambda_{\text{Ex/Em}} = 390/509$, a ubiquitous fluorescence peak in DOM in aquatic environments) was comparable to previous studies of groundwater DOM (average 0.06 ± 0.07) (Schittich et al., 2018; Wilson et al., 2023).

The second subgroup of samples (example shown in Fig. 1B) was dominated by fluorescence emission below approx. 420 nm. Samples in this subgroup showed a fluorescence peak around $\lambda_{\text{Ex}} = 250$ nm and $\lambda_{\text{Em}} = 340$ nm. These spectral characteristics and very high fluorescence levels do not agree with the occurrence of natural tryptophan-like or tyrosine-like fluorescence but closely resemble the properties of sulfanilic acid or sulfanilamide (Table 1, Fig. S2) present in the contaminant plume (Lakowicz, 2006; Wünsch et al., 2015; Birdwell and Engel, 2010; Coble, 1996). A query in OpenFluor (<http://openfluor.org>) did not yield matches with existing models of natural dissolved organic matter

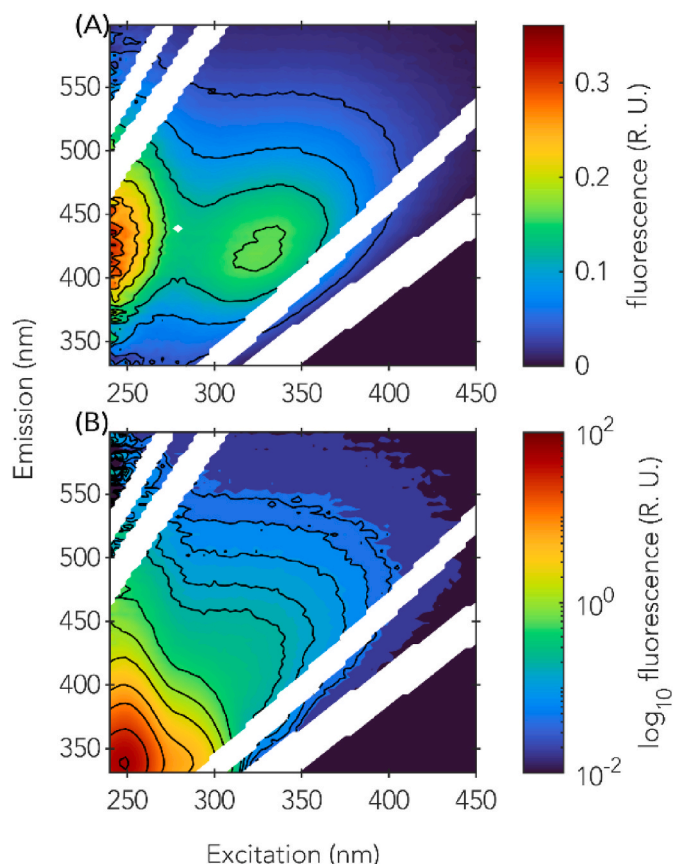


Fig. 1. Excitation-Emission matrices showing an example for uncontaminated groundwater consisting of natural FDOM (A) and groundwater contaminated with fluorescent compounds (B). The color scale in (B) was log-transformed to emphasize the presence of contaminants in addition to the natural background fluorescence.

(Murphy et al., 2014). OpenFluor contains spectra from more than 200 publications including natural and engineered and contaminated system. The lack of a match emphasizes the unusual nature of the fluorescence exhibited by samples at the Grindsted site (specifically Fig. 1B). Though similar, spectra associated with oils or pharmaceuticals did not yield significant matches either (Gou et al., 2017; Wasswa et al., 2019; Paradina-Fernández et al., 2023).

In fact, a strong correlation between $\sum_{\text{FSA}}$ and fluorescence emission in the ultraviolet range was observed (Fig. 2). Using the strong correlation, DOM fluorescence measured either via benchtop fluorometry or on-site sensors can be used as a surrogate to track the distribution of the sulfonamide plume. To that end, oil-targeting sensors ($\text{Ex} = 255$ nm), as well as protein-targeting sensors ($\text{Ex} = 275$ nm) present viable options to detect sulfonamides in the plume (see green and blue crosses in Fig. 2).

Interestingly, fluorescence in the visible range at $\lambda_{\text{Ex/Em}} 300/450$ also correlated well with $\sum_{\text{FSA}}$ ($R^2 = 0.85$ at $\lambda_{\text{Ex/Em}} 300/436$), see Fig. 2. None of the contaminants had significant fluorescence at $\lambda_{\text{Ex}} > 300$ nm and $\lambda_{\text{Em}} > 430$ nm and the contours in the visible wavelength range of the correlation matrix (Fig. 2) did not reflect spectra of contaminants. Hence, the correlation at $\lambda_{\text{Ex}} > 300$ nm and $\lambda_{\text{Em}} > 430$ nm was not directly tied to contaminants. Rather, a link between the occurrence of complex natural DOM and the contaminants is more likely. At present, the cause for this co-occurrence of natural DOM and anthropogenic contaminants is unknown and would require further experiments to investigate.

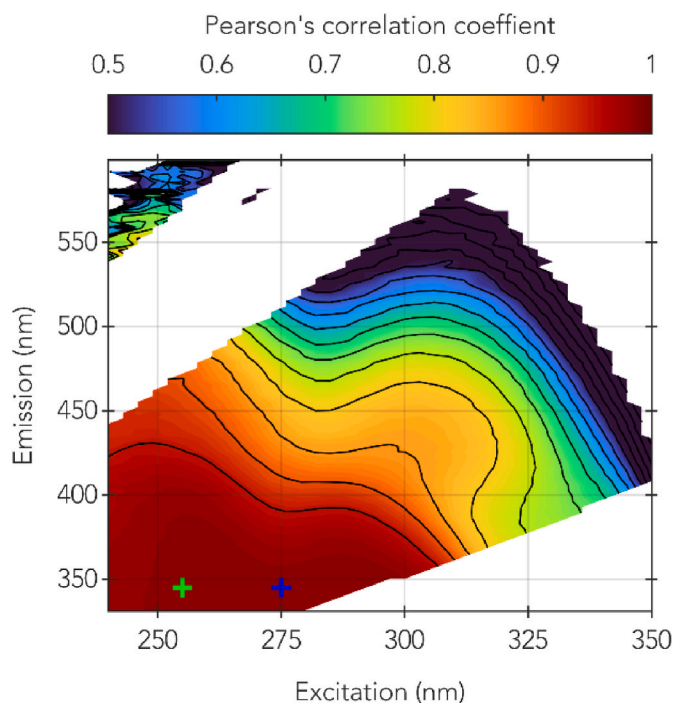


Fig. 2. Correlation matrix showing Pearson's r between Σ [sulfanilic acid, sulfanilamide and sulfaguanidine] and sample fluorescence at all possible excitation and emission wavelength pairs ($N = 140$). White areas represent missing data (scatter affected areas). The green and blue crosses represent the Ex/Em characteristics of two commercially available sensors that were tested in our study.

3.3. Limits of detection

The investigation of groundwater and pure compound fluorescence properties identified sulfanilamide, sulfaguanidine, and sulfanilic acid (Σ_{FSA}) as the most fluorescent surrogates for the tracking of the contaminant plume in Grindsted that also occurred at high concentrations. Next, the linear range and LOD for using fluorescence as a surrogate were quantified. These parameters were investigated for the benchtop spectrofluorometer, as well as the on-site sensor.

Thirty-five of the 140 field samples characterized with the benchtop spectrofluorometer contained less than $1 \mu\text{g/L}$ of Σ_{FSA} . An indication of contamination was observed for only one of these samples. In the range of $1\text{--}50 \mu\text{g/L}$, the majority (22 of 25 samples) showed fluorescence peaks clearly resembling sulfonamides and a linear relationship between Σ_{FSA} and the fluorescence intensity at Ex/Em 255/340 was found starting at concentrations around $10 \mu\text{g/L}$. All samples with more than $50 \mu\text{g/L}$ Σ_{FSA} showed clear signs of contamination (Fig. 3A) and fluorescence and Σ_{FSA} were significantly correlated ($R^2 = 0.97$, $p < 0.001$, root mean squared error relative to the average $\Sigma_{\text{FSA}} = 21\%$). For samples with more than 2 mg/L , the noise in the correlation with fluorescence was visibly larger. Beyond 8 mg/L , the fluorescence overestimated Σ_{FSA} . Thus, to establish the limit of detection of the benchtop fluorometer approach, a second linear regression was established with samples containing less than $2000 \mu\text{g/L}$ of Σ_{FSA} (Fig. 3B, $R^2 = 0.91$, root mean squared error relative to the average $\Sigma_{\text{FSA}} = 9.4\%$). Accordingly, the limit of detection of sulfonamides was $14 \mu\text{g/L}$. Above the LOD and cutoff fluorescence, six samples were spectrally dissimilar to sulfanilic acid (Fig. 3A). Difficulties in the detection of fluorescent contaminants with concentrations below $14 \mu\text{g/L}$ can arise due to the background fluorescence of natural FDOM, which can dominate the EEMs at low contaminant levels. Multivariate techniques such as PARAFAC would commonly be used to distinguish between different components and thus could help to distinguish contaminants from natural DOM, leading

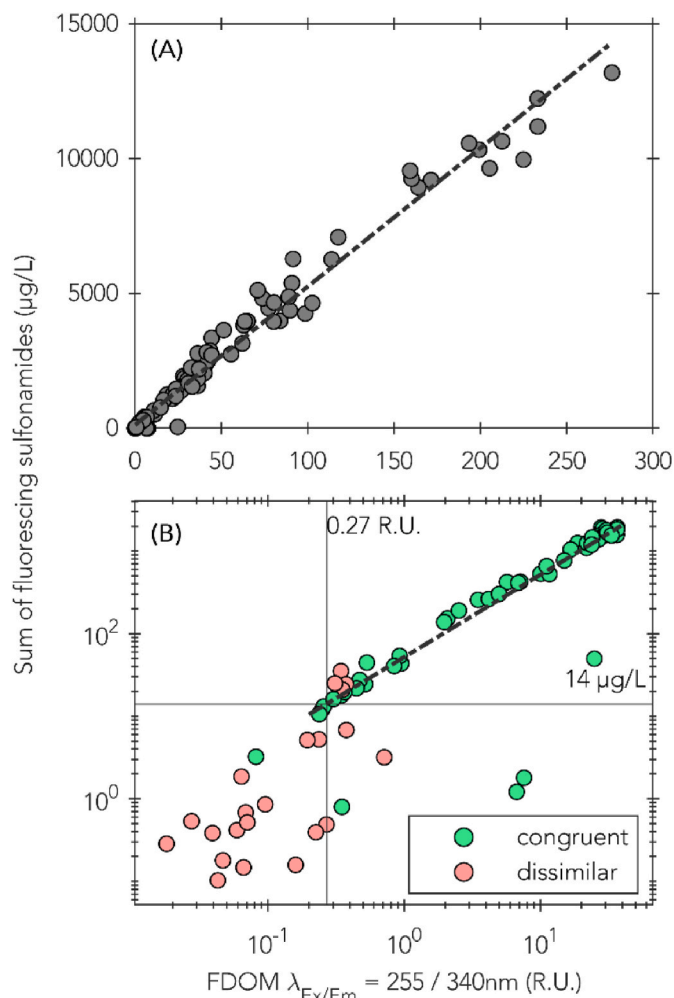


Fig. 3. Relationship between benchtop fluorescence readings and the sum of fluorescing sulfonamide concentrations. (A): All samples ($R^2 = 0.97$, $\text{RMSE} = 435 \mu\text{g/L}$). (B): Samples with Σ_{FSA} below 2 mg/L ($R^2 = 0.91$, $\text{RMSE} = 195 \mu\text{g/L}$). Colors reflect Tucker's congruence coefficient (spectral similarity) between the fluorescence emission of the sample and pure sulfanilic acid at $\lambda_{\text{Ex}} = 255 \text{ nm}$. Values above 0.95 indicate significant similarity (green dots), whereas lower similarity indicates significant non-specific (e.g.) natural fluorophores (red dots). Six of 140 samples had fluorescence higher than the LOD without spectral similarity to sulfanilic acid.

to LODs as low as $1 \mu\text{g/L}$ for selected pollutants (Paradina-Fernández et al., 2023). However, since the sulfonamides had highly similar fluorescence (see above), the assumption of spectral variability was not sufficiently satisfied, and we were unable to establish a robust PARAFAC model.

As mentioned above, the comparison between fluorescence and Σ_{FSA} showed six outliers where high fluorescence ($>0.27 \text{ R.U.}$) was detected although the targeted chemical analysis showed only low concentrations of sulfonamides in the samples (samples in the lower right corner of Fig. 3B). Since four of these samples showed a high similarity with sulfanilic acid fluorescence, the high levels of fluorescence are most likely tied to the contaminants in question and not cross-contamination. After careful consideration, the most likely explanation was sample mislabeling.

For 80 of the samples, a detailed analysis of the on-site data generated with the "refined fuels" sensor was then conducted. When comparing the on-site fluorescence observations with those conducted on the reference benchtop instrument (Fig. 4A), the linear sensor response above was limited to the sensor response below 2 V . Above 2 V , the sensor stopped responding to increases in fluorescence (Fig. 4A) and

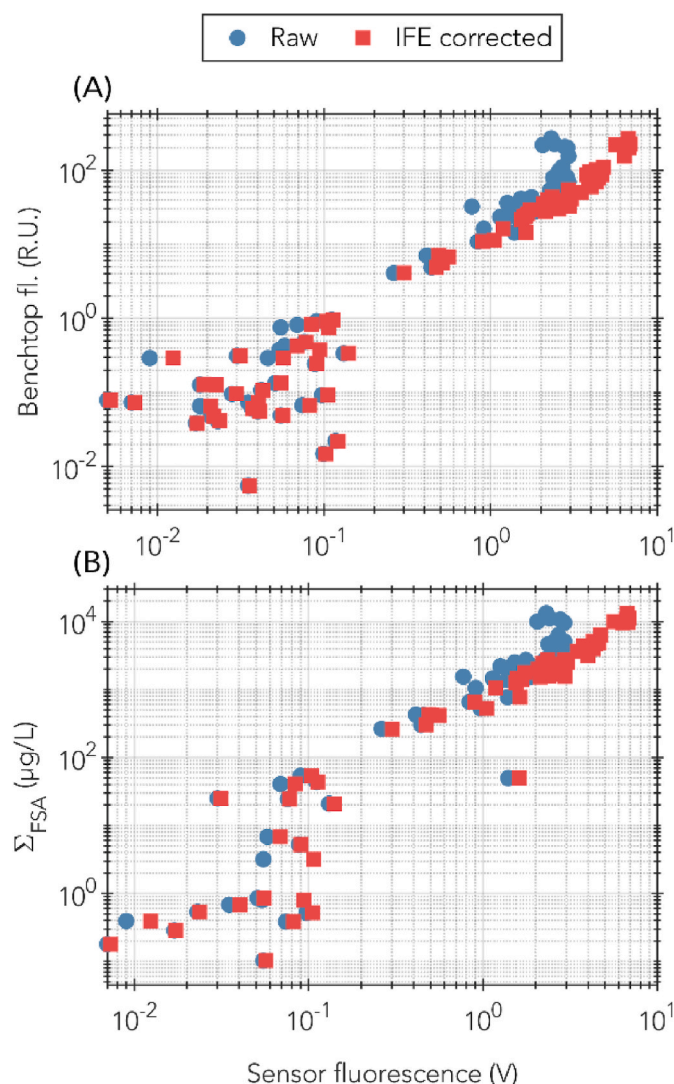


Fig. 4. Relationship between on-site sensor fluorescence and (A) Benchtop fluorescence; and (B) Sum of fluorescent sulfonamides (Σ_{FSA}). Blue filled circles indicate raw data 0–4.8V, whereas the red filled squares indicate hypothetical sensor responses where the bench top based absorbance was used to compensate for inner-filter effects.

increasing concentrations of Σ_{FSA} (Fig. 4B). This phenomenon was attributable to the inner filter effects (Eq. (2)) caused by the abundance of the intensely absorbing contaminants ($R^2 = 0.94$, $p < 0.01$ for the relationship between IFC and Σ_{FSA}) and was not related to the abundance of natural FDOM ($R^2 = 0.003$, $p = 0.69$ for the relationship between IFE and FDOM at $\lambda_{Ex} = 450$ nm and $\lambda_{Em} = 510$ nm). Upon compensation of the inner-filter effect with the sample absorbance, linearity for samples in the highly contaminated range was restored (Fig. 4A, red squares). These results highlight that the inner-filter effects in intensely contaminated samples can restrict the linear range of on-site sensors. However, as the main contribution of inner-filter effects stems from the absorbance at the excitation wavelength of 255 nm, a relatively inexpensive single-wavelength absorbance sensor could be used in future field studies to mitigate the issue and compensate for most of the non-linearity. This sensor should be designed to correct primary IFEs since these were responsible for over 95 % of the overall IFE in our case.

To establish a limit of detection for the on-site sensor, only the response range below 2V was considered to exclude non-linear responses due to IFEs (as established above). Though a significant correlation was observed ($R^2 = 0.8$, root mean squared error relative to

average $\Sigma_{FSA} = 54.8$ %), there was more noise in the relationship between sensor readings and sulfonamide concentrations when compared to the benchtop correlation (Fig. 5). Despite more abundant noise in the relationship, a clear correlation between Σ_{FSA} and fluorescence was apparent at concentrations above ~ 250 $\mu\text{g/L}$, whereas none of the samples below 50 $\mu\text{g/L}$ correlated well. Based on the data shown in Fig. 5, the LOD of the on-site sensor was estimated to be 142 $\mu\text{g/L}$.

The comparatively high LOD of 142 $\mu\text{g/L}$ in conjunction with a restricted linear range due to inner-filter effects highlights that the quantitative use of on-site sensors in highly contaminated water samples can be challenging. While this is less desirable than the lower LOD value for the grab samples or highly sensitive LC-MS techniques, the sensor can be used to effectively constrain the spatial extent of contaminant plumes at high sampling density due to fast and relatively easy measurements. Fig. 3B shows that for Σ_{FSA} , samples were either taken in the center of the plume and thus highly contaminated (>250 $\mu\text{g/L}$ Σ_{FSA}) or at the edge or outside of the plume (<50 $\mu\text{g/L}$ Σ_{FSA}). Since the LOD falls in between these two groups, the sensor can identify the highly contaminated regions of the plume during explorative campaigns. This should then be followed by systematic screening around these areas where the sensor can no longer provide a clear classification. Nonetheless, this screening process ultimately speeds up on-site screening and lower analysis costs, especially for large-scale investigations such as the one conducted at the Grindstedt site. Due to the relatively high LOD, the sensor contributes to such investigations but does not serve as a stand-alone tool. Building on our findings here, the on-site fluorescence sensor was used to quantify the mass transport of contaminants along with their vertical and horizontal distribution (Lemaire et al., 2024).

Though good correlations were established for both benchtop instrument and fluorescence sensors, a solid base knowledge about the plume composition is critical, especially regarding optically active compounds. The correlations shown in Figs. 3 and 5 only hold if the plume composition is relatively stable and no unknown compounds contribute significantly to the fluorescence at the sites. In our case, the three most fluorescent sulfonamides had median relative contributions of 1.8 ± 24.4 , 98.9 ± 22.4 , and 0.7 ± 25.3 % for sulfanilamide,

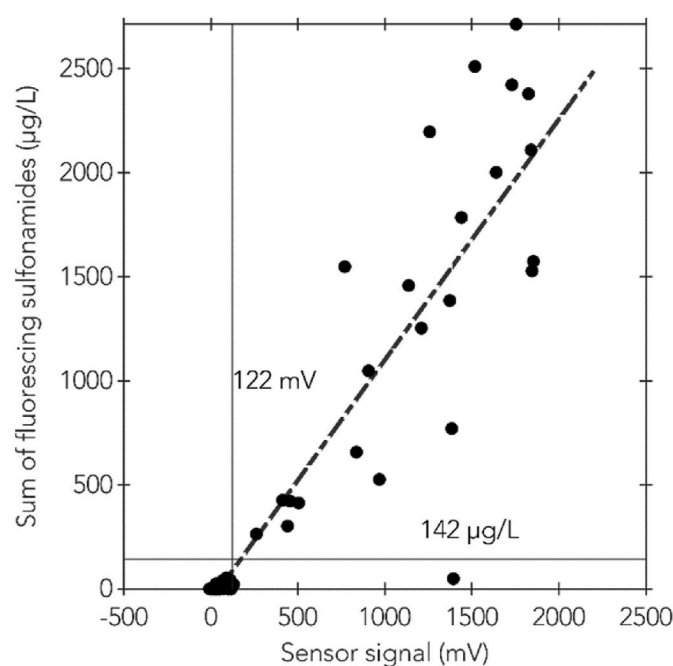


Fig. 5. Correlation between on-site sensor fluorescence and the sum of fluorescent sulfonamides (Σ_{FSA}). For the correlation, non-linear data with sensor responses above 2 V were excluded and samples with no detected Σ_{FSA} were excluded.

sulfanilic acid, and sulfaguanidine, respectively. While the plume thus was not constant in composition, the most contaminated samples were all dominated by sulfanilic acid and the correlation was thus not affected by varying composition. Nonetheless, the contribution of sulfanilic acid is known to differ spatially, e.g. the eastern and western part of the plume (forureningsundersogelse, 2023). This is especially important to consider since sulfanilic acid is not a sulfonamide and has no pharmaceutical effect itself, but is highly relied upon as an indicator for the plume. Unfortunately, due to the high similarity of optical properties, compositional changes were difficult to identify spectroscopically. To avoid misrepresentation, a proportion of samples should thus always be sent for targeted contaminant analysis for verification.

3.4. Can solid-phase extraction (SPE) increase the sensitivity?

For a solution of pure sulfanilamide at 100 $\mu\text{g/L}$, the PPL sorbent extracted the compound at an efficiency close to 100% for the first 20 mL of sample, whereas the efficiency decreased to approx. 40% at 50 mL (Fig. 6A, solid black line) with an average of 77% across the first 50 mL. Sulfanilic acid was not retained on the sorbent under the chosen conditions. The extraction efficiency of stream water DOM decreased fast to approx. 30 % after 25 mL (Fig. 6A, dashed black line) with an average of 37 % across the first 50 mL. The resulting enrichment factor of sulfanilamide (extraction efficiency of sulfanilamide divided by the extraction efficiency of DOM) exceeded 2.5 for approx. 20 mL and was 2.2 on average across the first 50 mL (Fig. 6A, red line).

To test whether enrichment is also achieved during a simultaneous extraction of sulfanilamide in a matrix of natural DOM, 100 mL of an uncontaminated stream water sample was enriched with 1 $\mu\text{g/L}$ sulfanilamide and the sample was extracted using the PPL sorbent. While no clear sign of sulfanilamide in the original sample was visible (Fig. 6B, red and black lines), a clear sulfanilamide peak was seen in the methanol extract (Fig. 6B, blue line). The selective enrichment of sulfonamide on PPL at circumneutral pH increased the peak prominence of sulfonamide by a factor of 3.4 from 0.16 to 0.55 ($\lambda_{\text{Ex/Em}}$ 250/341 relative to 250/447).

Though a direct comparison of the SPE experiment (Fig. 6) with field data (Fig. 3) with respect to LOD is difficult, the increased prominence would likely not elevate the sulfonamide fluorescence above the LOD required for bulk analyses as shown in Fig. 3. In the field samples, the

peak prominence as calculated above was between 0.5 and 3 for samples with $15 \pm 5 \mu\text{g/L} \sum_{\text{FSA}}$. Nonetheless, conducting small-scale SPEs could certainly lower the LOD with the aim of discriminating between contaminated and uncontaminated samples to guide sampling for expensive in-depth LC-MS quantifications. Since the most prominent enrichment took place at volumes below 50 mL, volume requirements are practically identical to the common protocols for fluorescence samples. By staying at circumneutral pH and low sample volumes, the addition of the SPE extraction requires only small investments of time and resources. However, the future implementation of an SPE approach will certainly require further testing of commercially available sorbents to achieve an extraction of *all* relevant contaminants (not only sulfanilamide). It is highly likely that a higher enrichment factor and more quantitative results could be obtained by adequately adjusting the pH of the sample to match the characteristics of the selected sorbent and by measuring the extract fluorescence in water instead of eluent solvent. However, as these steps add complexity, a careful investigation of costs and benefits associated with the SPE enrichment should be conducted in a future study.

4. Final remarks and future directions

A field study of a contamination plume (Grindsted, Denmark) revealed the presence of several pharmaceuticals that fluoresced strong enough to be detectable at environmentally relevant concentrations. For the sum of sulfanilamide, sulfanilic acid, and sulfaguanidine, the LOD was 14 $\mu\text{g/L}$ using a benchtop spectrofluorometer. When a sensor was deployed on-site, the LOD was 142 $\mu\text{g/L}$ and we identified inner filter effects to be a limiting factor for reliable quantification at high concentrations. At the lower end, the co-occurrence of natural DOM and contaminants made quantification difficult since the contaminant fluorescence was overpowered by natural DOM. The otherwise common application of machine learning (PARAFAC) was not feasible due to the high degree of spectral similarity between several of the contaminants at the Grindsted site.

For freshwater environments with low natural DOM concentrations, such as ground- or drinking water, fluorescence monitoring thus represents a promising screening approach for the investigated contaminants due to the relatively low level of natural fluorescence. With a sharp gradient from uncontaminated to heavily contaminated samples

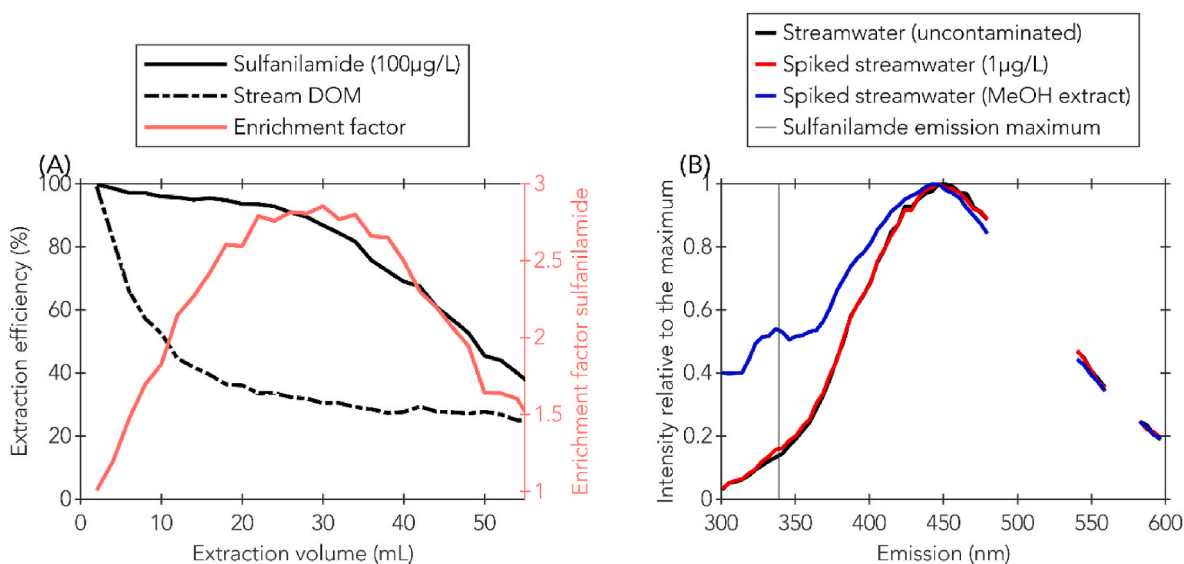


Fig. 6. Extraction performance of the PPL sorbent. (A): Extraction efficiencies for sulfanilamide (black, solid line) and natural DOM (black dashed line) as a function of extraction volume. The preferential enrichment of sulfanilamide over DOM is shown for reference (red solid line). (B): Pre- and post-extraction emission spectra ($\lambda_{\text{Ex}} = 250 \text{ nm}$) of stream water (black), stream water spiked with 1 $\mu\text{g/L}$ sulfanilamide (red), and the methanol extract of the spiked stream sample after extraction of 100 mL (blue). The black vertical line indicates the emission maximum of sulfanilamide, $\lambda_{\text{Em}} = 250 \text{ nm}$.

at our field site, the on-site sensor was a valuable tool to distinguish between contaminated and uncontaminated samples despite a relatively high LOD. In order to quantify contamination in highly contaminated samples, our work demonstrates that primary inner-filter effects could be compensated via an additional, relatively inexpensive single-channel absorbance sensor. The deployment of on-site sensors during field campaigns can inform the sampling strategy in real-time, subsequently reducing costs for advanced analyses by lowering the sample numbers. We have recently successfully demonstrated the utility of the on-site sensor discussed here for the mass-discharge estimation at the Grinds- ted site (Lemaire et al., 2024).

In future campaigns, the sensor may be built into an in-situ probe similar to the *Ultra Violet Optical Screening Tool* (UVOST) or the *Optical Image Profiler* (OIP) for direct measurements during drive point sampling. Such a sensor could help to identify locations for water sampling as a pre-screening tool, supplemental to (more expensive) LC-MS based investigations. For water matrices with high levels of natural background fluorescence, such as wastewater or recharged aquifers, fluorescence-based contaminant screening likely represents a greater challenge. However, even for such complex matrices, fluorescence can serve as a sensitive monitoring technique when deployed in conjunction with spectral decomposition routines (Paradina-Fernández et al., 2023).

Many anthropogenic contaminants are aromatic compounds, which theoretically might have the ability to fluoresce. With promising results across a number of exploratory studies, there is an urgent need for a centralized database of contaminant absorbance and fluorescence. The existence of a central database would allow scientists and managers to easily evaluate whether fluorescence screening (either benchtop instruments or field sensors) might be an applicable tool in their specific case. Additionally, the identification of unknown fluorescence phenomena would be far easier.

CRedit authorship contribution statement

Laila Vinther: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Mette M. Broholm:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Anna-Ricarda Schittich:** Writing – review & editing, Supervision, Methodology, Investigation. **Therese Haugsted:** Writing – review & editing, Validation, Investigation. **Ursula S. McKnight:** Writing – review & editing, Validation, Supervision, Project administration, Conceptualization. **Helene Draborg:** Writing – review & editing, Validation, Investigation. **Poul L. Bjerg:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Urban J. Wünsch:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was in part funded by the Region of Southern Denmark. The authors would like to thank laboratory and field staff at DTU and Rambøll for assistance with collecting and analyzing field samples. We extend our gratitude to Cecilie H. H. Hansen for contributing to the initial investigations.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2024.144009>.

<https://doi.org/10.1016/j.chemosphere.2024.144009>.

Data availability

Fluorescence EEMs and contaminant concentrations along with sensor fluorescence values are available at <https://doi.org/10.11583/DTU.28106633>. Spectra of the pure contaminants will be available in the OpenFluor database (<http://openfluor.org>).

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