Lab to Lake: Excitation-Emission Matrix's Voyage from Theory to Practice

ラボから湖へ：励起発光マトリックスの理論から実践への航海

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Dissolved organic matters (DOMs) significantly impact water quality and contaminant behavior, making their monitoring essential for water treatment. Excitation-emission matrix (EEM) fluorescence spectroscopy is a powerful tool for this purpose but faces challenges such as complex fluorescence property of DOM, turbidity interference, and limitations in field applications. To address these challenges, we developed an advanced algorithm based on the charge transfer model for accurate DOM decomposition and a new method to reduce scattering interference, improving EEM accuracy. Additionally, we introduced a sparse EEM reconstruction algorithm, leading to a miniaturized, portable device for real-time, in-situ monitoring. This innovation enhances the feasibility of EEM spectroscopy in field monitoring, contributing to efficient, precise water quality assessments and supporting sustainable water management practices.

溶存有機物(DOM)は水質と汚染物質の挙動に大きな影響を与えるため，水処理にはその監視が不可欠である。励起発光マトリックス(EEM)蛍光分光法は，この目的のための強力なツールであるが，DOMの複雑な蛍光特性，濁度干渉、現場での適用における制限などの課題に直面している。これらの課題に対処するために，正確なDOM分解のための電荷移動モデルに基づく高度なアルゴリズムと，散乱干渉を低減してEEMの精度を向上させる新しい方法を開発した。さらに，スパースEEM再構成アルゴリズムを導入し，リアルタイムの現場監視のための小型でポータブルなデバイスを実現した。この革新により，現場監視におけるEEM分光法の実現可能性が高まり，効率的で正確な水質評価に貢献し，持続可能な水管理の実践につなげていく。

Introduction

Dissolved organic matter (DOM), a heterogeneous mixture of polysaccharides, proteins, peptides, humic substances, and lipids, is pervasively present in waters and serves as a key environmental indicator[1],[2]. Containing various aromatic chromophoric groups such as phenols, quinones, and indoles, DOM significantly alters the water‘s hue, engaging as both reactants and mediators in hydrochemical processes[3],[4]. The diverse functional groups enable the DOM-pollutants interactions, thus influencing their properties, transportation, transformation, and ultimate fate[5],[6]. Due to the pivotal roles of DOMs in the migration and transformation of pollutants and the formation of disinfection by-products, an efficient monitoring of their contents and composition is of vital importance for better comprehension of aquatic environments and improving the water treatment process[7],[8].

Ultraviolet-visible (UV-Vis) and fluorescence spectroscopies are particularly adept at characterizing DOM’s aromatic groups, which are linked to molecular weight distribution, hydrophilicity and hydrophobicity, reactivity, and concentration quantification[9],[10]. These techniques, sensitive to the absorption or emission of electromagnetic radiation, are thereby unveiling DOM’s intrinsic properties in diverse chemical environments[11],[12]. Compared to UV-Vis spectra, fluorescence spectra can identify more nuanced changes in DOM’s structure or composition by detecting peaks located at different excitation or emission wavelengths[13],[14].

With advancements in fluorescence spectrometry, it’s now possible to generate excitation-emission matrix (EEM) fluorescence spectra that offer richer information by collecting emission spectra at a series of excitation wavelengths. EEM

heralded for its rapidity, precision, selectivity, and depth of insight, has been instrumental in delineating the migration dynamics and transformation mechanisms of DOM[15],[16]. However, it is very challenging to adapt such technologies from controlled laboratory settings to the monitoring of complicated natural waters, because the intricate fluorescence behaviors of DOM impede the accurate interpretation of EEM spectra. Moreover, the presence of turbidity in water samples substantially hampers precise spectral analysis. Additionally, the prohibitive costs and rigorous operational prerequisites of conventional EEM spectrometers further confine their usage predominantly to laboratory investigations. These drawbacks have severely restricted a broader application of the EEM spectroscopy in environmental surveillance.

To address these challenges, we have investigated into the intricate fluorescence mechanisms of DOM, and based on which optimized the EEM analytical algorithms for enhanced speed and accuracy and enabling miniaturization of EEM spectrometers. Initially, we developed an algorithm for the precise decomposition of the EEM of DOM, based on the charge transfer model. Subsequently, we established a model to deduce the absorption spectrum from Rayleigh scattering, grounded in the principles of light scattering. Leveraging these spectral analysis enhancements, we engineered a compact EEM pollution traceability device. This innovation provides essential technological and instrumental support for implementing EEM spectroscopy in the real-time surveillance and identification of pollutants within aquatic environments.

Non-trilinear independence nature of DOM's EEM

The Parallel Factor Analysis (PARAFAC) is one of the most popular methods for DOM characterization. it decomposes the original dataset composed of a series of EEMs into several linear independent components (Figure 1A)[17],[18]. Due to the rapidity and plentiful information obtained from PARAFAC, it has become the most popular approaches and widely be applied in analyzing EEMs with complex unidentified components, such as DOM from various sources. However, PARAFAC decomposes the EEMs mathematically and cannot provide a physical interpretation for each component. Thus, the number of factors and their explanations are usually further clarified through methods like peak-picking and fluorescence regional integration. This problem occurred because PARAFAC assumed that the complex EEM of DOM are a linear superposition of fluorescence signals from independent fluorescent groups. However, environmental monitoring data have revealed that DOM, especially its major components like humic and fulvic acids, do not conform to this assumption, resulting in spectral distortion and errors in quantitative results. This limitation arises from the fact that PARAFAC is only applicable when the excitation and emission spectra are independent, making it unsuitable for scenarios involving charge transfer processes.

The charge transfer model is a fluorescence mechanism model that describes the transfer of electrons from donor molecules to acceptor molecules. This model emphasizes the importance of the electron transfer process in the fluorescence behavior of dissolved organic matter, especially in complex molecules containing both electron donors and acceptors. Compared to the basic principles of the traditional fluorescence model, the charge transfer model exhibits significant differences. Traditional fluorescence models focus on electron transitions within the molecule, assuming that fluorescence emission is solely related to changes in the molecule's excited state energy levels, typically applicable to simple organic molecules. In contrast, the charge transfer model posits that electrons not only transition within the molecule but can also undergo intermolecular charge transfer, a process that significantly impacts fluorescence intensity and wavelength.

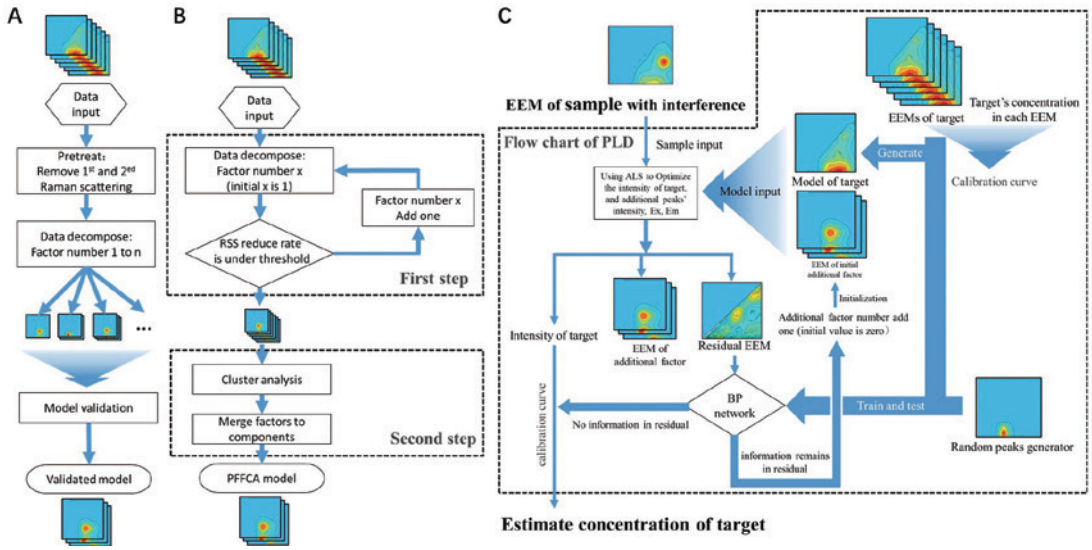

Figure 1 Workflow of (A) Parallel Factor Analysis (PARAFAC), (B) Parallel Factor Framework with Cluster Analysis (PFFCA) and (C) Prior Linear Decomposition (PLD).

Due to these mechanistic differences, the charge transfer model is particularly applicable to molecules with complex structures and electron donor-acceptor functionalities, such as dissolved organic matter like humic acid and fulvic acid[19]. Traditional fluorescence models fail to adequately describe the fluorescence emission of such substances, particularly in complex environments, where the fluorescence behavior of dissolved organic matter is significantly influenced by environmental factors and the charge transfer process. Therefore, the peak attribution results from the analysis method based on traditional fluorescence model can sometimes vary, potentially causing confusion or misleading the accurate identification of DOM components.

Analyze the EEM of DOM with a charge transfer model

The charge transfer model provides a more complex framework for a deeper understanding of the fluorescence characteristics of these dissolved organic compounds in real-world environments. Therefore, it should have important applications in environmental science fields such as water quality monitoring and pollutant tracking. However, traditional fluorescence analysis methods are based on the traditional fluorescence model, lacking consideration of the charge transfer process. This limitation can lead to inaccurate or constrained analysis when dealing with DOM.

Therefore, we simulated the EEM of DOM under conditions of charge transfer, and developed a novel method integrating the parallel factor framework with cluster analysis (PFFCA) [20]. The PFFCA method principally assumes that all of the components are independent and that the dataset is decomposed into many factors without considering charge transfer model. Afterwards, the factors whose scores are highly correlated was combined into one component because they may individually represent only a proportion of the component due to the charge transfer (Figure 1B). Therefore, in the first step, EEM dataset was decomposed into a set of trilinear terms and a residual array using the PARAFAC model. The factor number was determined when the residual was not decreased with the increasing of the factor number. This process is based on the idea that if the increase in factor number cannot reduce the residual, the increase is no longer necessary.

For the second step, PFFCA clusters these factors into several practical DOM components by calculating the coefficient of the scores from different components. If the scores of several factors correlate consistently with each other, they are likely derived from the same DOM component due to the charge transfer. Consequently, both trilinear and non-trilinear DOM fractions can be accurately identified. This approach, by decoupling the data decomposition and analysis processes, eliminates the impact of non-linear superposition of fluorescent groups on the analysis. PFFCA achieves stable and interpretable information on DOM components by clustering the variation patterns of different factors with disturbances.

This work represents a breakthrough from the traditional understanding of linear superposition assumed by PARAFAC, reversing the common practice of introducing errors from non-trilinear components in quantitative analysis. Compared to traditional methods, PFFCA can more accurately reflect the composition of DOM in water (with a reduction in the sum of squared residuals of the spectra by at least an order of magnitude), providing a reliable method for analysis of the DOM components. For the quantification applications, the scores from standard solution samples can also be linearly fitted with concentration vectors to determine their contributions. Then, the unknown concentration samples' scores are calculated with the EEMs of each component, followed by concentration calculations via linear regression[21].

Despite that the new method partially overcome the obstacles of PARAFAC, the analysis procedures are relatively time-consuming, due to the reserve of PARAFAC framework and the subsequent cluster analysis or multiple regression fitting. Therefore, we optimized the PFFCA by reevaluating the independence of data, thereby refining the linear iterative approach[22]. This prior linear decomposition (PLD) method firstly involves linear regression of sample EEMs with the standard solution, followed by residual analysis via a backpropagation artificial neural network. If there are still additional peaks in the residual, the factor number will be increased by one until a residual EEM with random noise is obtained (Figure 1C). The final concentrations of the target and the EEMs of the additional factors will be simultaneously estimated, allowing PLD to quantify the DOM and diagnose potential fluorescent interfering substances. By incorporating known EEMs as prior knowledge, the optimization process transcended the limitations of the linear superposition model. Furthermore, integrating machine learning enabled rapid alerts for abnormal fluorescence peaks without human intervention, significantly reducing analysis time from PFFCA's 30 minutes to less than 40 seconds. Our method revolutionizes the traditional regression-based "needle in a haystack" workflow for estimating unknown pollutants, providing a new approach for faster, more interference-resistant EEM analysis. Leveraging these innovative algorithms, we developed an EEM-based method for detection of non-fluorescent saccharides[21], enabling simultaneous quantification of aldoses and ketoses, and optimized the selection of protein standards, thereby expanding the scope of EEM's application in environmental monitoring.

Eliminates scattering interference in turbid water samples

Spectral analysis methods typically require samples to be clear, true solutions. However, the presence of particles in actual water environments is inevitable and can cause turbidity, posing challenges to practical environmental monitoring application. Conventional spectral testing often necessitates preprocessing steps like centrifugation or membrane filtration to remove particulate interference. However, these processes not only significantly restrict the development of in-situ water environment spectral monitoring techniques but also risk omitting crucial environmental information since these particles themselves could be pollutants. Therefore, a thorough understanding of the light scattering behavior of particles in the environment is crucial for accurately interpreting the environmental spectral data.

EEM spectroscopy naturally includes Rayleigh scattering signals, where the excitation wavelength equals the emission wavelength. However, traditional analysis methods often cannot resolve complex scattering signals and thus typically set these scattering signals to zero to avoid interference with EEM analysis. Based on the deep understanding of light scattering and EEM, we develop our estimation method based on the following two hypotheses:
obedience to Beer's law ($I=I_0e^{-\alpha_{ext}h}$), and the far smaller illuminated area than the cuvette cross-section. By applying Beer's law, the strength of the Rayleigh scattering signal was given as (Figure 2A):

$$S(\lambda) = I_0 \int_{x_1}^{x_2} \alpha_{sca}(\lambda) e^{-(\alpha_{sca}(\lambda)+\alpha_{abs}(\lambda))x}\, dx \cdot k_{\frac{\pi}{2}}(\lambda) \int_{y_1}^{y_2} e^{-(\alpha_{sca}(\lambda)+\alpha_{abs}(\lambda))y}\, dy \quad (1)$$

where I_0 refers to the incident light intensity while $\alpha_{sca}(\lambda)$ and $\alpha_{abs}(\lambda)$ represent the coefficients of Rayleigh scattering and absorption[23] with the unit of cm^{-1}, their sum is the attenuation coefficient α_{ext} in Beer's Law. Meanwhile, $k_{\frac{\pi}{2}}(\lambda)$ is the proportion of Rayleigh scattered photons detected by the right-angle observation[24] sensor to all Rayleigh scattered photons in the region of integration.

For the second hypothesis when the illuminated area is far smaller than the cuvette cross-section, the approximation of eq. 1 can be given as:[25],[26]

$$S(\lambda) = \alpha_{sca}(\lambda) k_{\frac{\pi}{2}}(\lambda) I_0 (x_2 - x_1)(y_2 - y_1) e^{-(\alpha_{sca}(\lambda)+\alpha_{abs}(\lambda))(C_{ex}+C_{em})} \quad (2)$$

where x_1, x_2, y_1 and y_2, expressed in two-dimensional Cartesian coordinates, define the boarder of illuminated area in the top view of a cuvette. Moreover, C_{ex} and C_{em}, representing midpoint coordinates of the optical path in cuvette along the x and y axis in an ordinary circumstance[26]. As a result, eq. 2 could be rewritten as:

$$S(\lambda) = \gamma(\lambda)\alpha_{sca}(\lambda) e^{-(\alpha_{sca}(\lambda)+\alpha_{abs}(\lambda))} \quad (3)$$

where $\gamma(\lambda)$ is the multiplication of $k_{\frac{\pi}{2}}(\lambda)$, I_0, $x_2 - x_1$, and $y_2 - y_1$ in eq. 2 that can be seen as a constant. With the blank control of the sample written as $S_{blank}(\lambda) = \gamma(\lambda)\alpha_{sca}(\lambda) e^{-\alpha_{sca}(\lambda)}$, a deduction can be made to estimate the absorbance of the sample by introducing a relationship of $\alpha_{abs}(\lambda) = ln(10) \cdot A(\lambda)$ for any cuvette with 1 cm light path:

$$A(\lambda) = log_{10}\left(S_{blank}(\lambda)\right) - log_{10}\left(S_{sample}(\lambda)\right) \quad (4)$$

where A(λ) is the absorbance of sample in wavelength λ, S_{sample} and S_{blank} are Rayleigh scattering signal strength for sample and blank control detected under the same configuration of measurement (Figure 2B). Therefore, the estimated absorbance can be used to calibrate the inner filter effects in EEM measurement, overcoming the bottleneck that EEM requires additional light sources or detectors for accurate quantification. Our method provides a theoretical basis for the optimization of fluorescence spectroscopy instrument optical paths and offers methodological support for the structural optimization of EEM monitoring instruments in actual water environments[27].

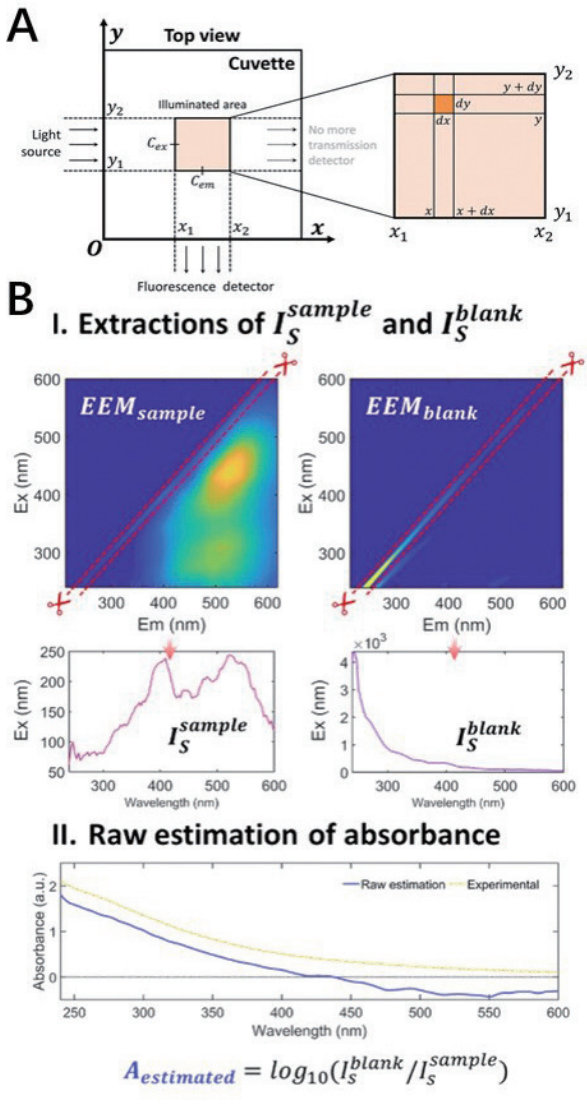

Figure 2 (A) The geometry of infinitesimal analysis in the model. (B) Workflow of the absorbance estimation with the Rayleigh scattering.

In aquatic environments, the adsorption and distribution of DOM on particles largely affect the migration and transformation of both DOM and pollutants. However, such processes can result in organic matter concentrations exceeding the linear range of the Beer-Lambert law. Thus, traditional analysis methods based on radiative transfer theory, which decouple scattering and absorption spectra, often lead to spectral distortions and fail to quantitatively resolve the distribution process. Leveraging a new understanding of the scattering model, we established a distribution model for the adsorption process of environmental particles. This model corrected for the nonlinear changes in adsorption spectra with concentration, and analyzed the distribution process, proportion, and rate of pollutants in water and on particle surfaces. Using this method in conjunction with infrared spectroscopy, we elucidated how the DOM interacts through π-π conjugation with polystyrene microplastics containing benzene rings and large condensed domains, providing essential support for analyzing the migration behaviors of pollutants carried by microplastics in actual environments [28].

Development of miniaturized EEM monitoring device

Although we have deciphered the EEM of DOM in actual environmental water samples, transitioning the EEM spectrometer from laboratory analysis to in-situ monitoring in the field introduces new challenges such as harsh and variable environments, the need for low power consumption, and the requirement for long-term stability with minimal maintenance. Therefore, due to the short lifespan of mercury/xenon lamps and the high sensitivity of monochromator to temperature and vibrations, the traditional EEM spectrometers struggles to cope with the complex and harsh conditions of actual monitoring scenarios. Although light-emitting diodes (LEDs) offer longer lifespans and stable performance, their poor monochromaticity and discontinuous spectra make obtaining comprehensive spectral information challenging due to spectral sparsity.

To address this issue, we introduced the manifold embedding hypothesis, theoretically proving that, under appropriate wavelength selection, sparse spectra collected with LEDs contain equivalent information to continuous spectra. Based on this, we developed a deep learning network with an encoder-decoder architecture, creating a method for the continuous reconstruction of sparse spectra. This approach allows the use of LED light sources in EEM spectrometers, breaking free from the constraints of traditional mercury/xenon lamp-monochromator structures and providing methodological support for the miniaturization of EEM spectrometers.

Building on the innovations in analytical algorithms, we developed a portable water quality monitoring and traceability device based on EEM spectroscopy. Compared to existing EEM spectrometers that are mainly confined to lab use, this device not only maintains the quality and quantitative accuracy of EEM but also offers advantages such as interference resistance, compact size, extended lifespan, and rapid testing. Therefore, we integrated this device onto autonomous surface vehicle (ASV) for high-density navigational monitoring of the Chao Lake, China's third-largest freshwater lake (Figure 3). It enabled monitoring changes in water quality as tributaries merge, and identifying potential sources of wastewater discharge or leaks through the abnormal fluctuations in EEM components. With the new method powered EEM spectrometer, we have provided technical support for the management and regulation of the Chao Lake.

In addition, EEMs with high spatial and temporal density can also provide sufficient data support for big data analysis of the water environment. For example, only with dense spatiotemporal data can we unveil whether environmental variables show gradual or abrupt changes over time and space. Therefore, we conduct a field tests covered 8.3 km of the Nanfei River in Hefei, China provided 132 samples with the spatial resolution less than 63 m. With these data, we could use the noise color [29]-[31] to explore the structure of spatial autocorrelation of fluorescent noise along the Nanfei

Figure 3 Homemade compact EEM spectrometers

River, which is impossible for the manual EEM data collection. Noise color, informed by power spectrum density[29], reflects the frequency variation of a variable. This allowed us to distinguish between fluorescence components that exhibit low-frequency, spatially continuous changes, and those with high-frequency variations linked to specific geographical locations.

Predominantly, white noise represented about 83% of the EEM spectral area, indicating random variation in fluorescence, particularly in the coordinates of humic-like substance (Figure 4). However, pink noise was primarily associated with the T peak of protein-like substance, indicating a smoother variation of protein-like substances along the river. These findings imply that humic-like substance exhibits relative local heterogeneity, while protein-like substance demonstrates a more uniform distribution within the river, indicating that the differing origins of humic-like and protein-like substances play a crucial role. Thus, the miniaturized EEM monitoring device has the potential to enrich our comprehension of water variations.

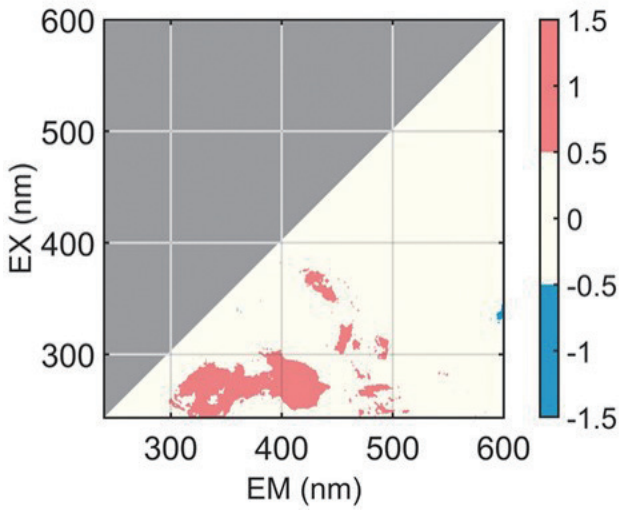

Figure 4 Noise colors of elements in EEM across 132 ASV sampling points.

Conclusion

By addressing the intricate challenges of accurate DOM analysis in natural waters, we made advancements in EEM analytical algorithms and instrumental innovation, enabling EEM spectroscopy applied to actual environmental monitoring. The development of the algorithm based on the charge transfer model for precise DOM decomposition, and spectral analysis method to eliminate scattering interference, fundamentally enhances the accuracy and practical applicability of EEM spectroscopy. Furthermore, we successfully miniaturized the EEM spectrometer, culminating in a compact, portable device for real-time environmental surveillance, representing a significant leap towards practical, field-deployable solution. This work not only bridges the gap between sophisticated laboratory analyses and the exigencies of in-situ environmental monitoring, but also opens new avenues to assist the enforcement of sustainable water management practices. By enabling more precise, efficient, and accessible monitoring of water quality, these works offer profound implications for environmental science, water treatment technologies, and policy-making, aligning with the global imperatives of environmental conservation and public health.

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