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Establishing a Raman spectra database for the detection of Terbuthylazine metabolites using SERS-based optical sensors

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Summary:

This study presents a comprehensive theoretical and experimental examination of Raman spectra for prevalent pesticide metabolites in Danish groundwater. The objective is to facilitate sensor development and enhance chemical identification processes by creating a database for the Raman spectra of terbuthylazine metabolites using three excitation wavelengths.

Keywords: groundwater, terbuthylazine, metabolites detection, Raman spectroscopy, Density Functional Theory.

Background, Motivation, and Objective

Climate change significantly impacts hydroclimatic events across Europe, leading to an increase in the frequency and intensity of extreme weather phenomena such as heavy rainfall, prolonged droughts, and heat waves. These hydroclimatic events play a crucial role in influencing the behavior and distribution of pesticides in the environment [1]. They can substantially affect the transport, degradation, and persistence of pesticides, often resulting in increased contamination levels in both surface water and groundwater. Understanding the relationship between hydroclimatic conditions and pesticide dynamics is essential, especially as climate change is expected to intensify extreme weather events. Many pesticides lack established Environmental Quality Standards (EQS) due to insufficient data on their concentrations and ecological effects. [2]. Collecting pesticide occurrence data will help standardize EQS and safeguard water quality within the EU's evolving regulatory frameworks. In Denmark, many banned or restricted pesticides still appear in groundwater, often exceeding the 0.1 µg/L threshold. For example, terbuthylazine, banned since 2008, has its breakdown products detected at frequencies up to 70% and concentrations ranging from 0.01 ng/L to 9.7 µg/L. The highest concentration was found for terbuthylazine-TP MT23 (LM5) while the lowest was for terbuthylazine-TP MT24 (LM1) [3]. This persistence underscores the need for improved detection methods.

Raman spectroscopy is an effective analytical technique for detecting pesticides, offering distinct spectra for identification and quantification. Its main advantage is efficiency, with analyses often completed in minutes, enabling rapid screening of large sample sets, crucial during contamination events or routine monitoring. Surface-Enhanced Raman Spectroscopy (SERS) has been effectively used to analyse different pesticide classes. Its miniaturization on a chip allows for compact sensors that enable sensitive on-site analysis [4]. This study examines the Raman spectra of common metabolites found in Danish groundwater. The objective is to facilitate sensor development and enhance chemical identification processes by creating a database for the terbuthylazine metabolites Raman spectra using three excitation wavelengths.

Simulation and detection

Density Functional Theory (DFT) calculations were carried out using Gaussian 16 to simulate the Raman spectra of the target compounds.

The calculated spectra were compared with experimental Raman data to validate the vibrational modes and identify characteristic peaks. Each prominent spectral feature was analyzed in terms of the corresponding vibrational motion and the specific part of the molecule responsible. Theoretical insights provided by the DFT simulations enhanced the interpretation of the experimental spectra, enabling accurate assignment of functional groups.

The samples of metabolites prepared for Raman analysis were created using gold-coated silicon substrates, which were structured with defined spotting areas to ensure structural integrity through the application of pressure-sensitive adhesive (PSA). Following the drop-casting of 7 µL of the metabolite solution, the samples were covered with a glass substrate to serve as a protective layer. The prepared solutions consisted of various terbuthylazine metabolites, which were diluted in water to concentrations of 10^{-3},10^{-4} mM, starting from a stock solution of 10 mM that was prepared in ethanol, which subsequently was evaporated, and the compounds were dissolved in distilled water.

Raman spectra for 532nm and 633nm were obtained utilizing a Horiba LabRAM HR Evolution Spectrometer, employing a 50x objective lens at maximum power (17 mW). The spectra were recorded by directing the laser through glass onto the metabolite crystallites present on the analyzed substrates, with an acquisition time of 5'' and 5 accumulations, with a total time of 4'56'' per sample. Raman spectra for 785nm excitation wavelength were collected using BWS465-785S i-Raman Plus spectrometer. The spectra were collected for 240'', 2 accumulations and 306mW of power.

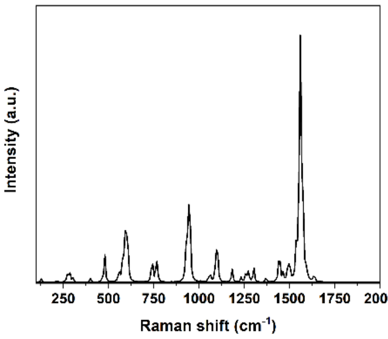

Fig. 1. Terbuthylazine-TP MT23 (LM5) simulated Raman spectra using DFT calculation

Results

For all the involved metabolites, we extracted and compared the simulated spectra with the experimental Raman spectra. For instance, in the case of terbuthylazine-TP MT23 (LM5), which is the most abundant metabolite, the spectra are presented in Fig. 1 and 2. The experimental spectra have been normalized for better clarity. We chose the LM5 metabolite not only for its frequent detection but also because its spectra vary significantly with the excitation wavelength.

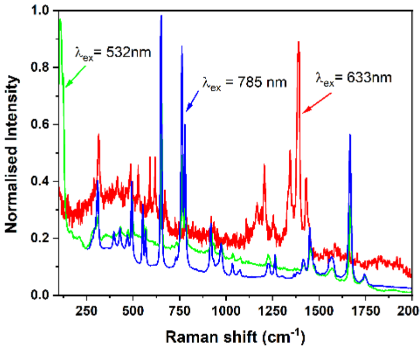

Fig. 2. Terbuthylazine-TP MT23 (LM5) experimental Raman spectra

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