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RAPID EVALUATION OF GROUNDWATER QUALITY BASED ON FLUORESCENCE RATIOS

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Abstract. *Water quality is influenced by the content of dissolved organic matter (DOM). For this study, water samples were collected from a rural area predisposed to contamination due to either intensive agricultural activities or to the faulty management of animal and human waste disposal. Fluorescence spectroscopy was used for analyzing the degree and the possible source of contamination of the water sources. Excitation–emission matrices (EEMs) were presented, evidencing specific fluorescent fingerprints characteristic to clean, drinkable water and to contaminated water. In the case of the samples for which contamination was established, the type of organic matter input, either humic or proteic, was determined. For the samples where proteic input was confirmed, the source of the contamination was evidenced. A thorough analysis of fluorescence intensity ratios obtained for only one excitation wavelength, facilitated a better identification of the type and source of water contaminant.*

Keywords: Dissolved organic matter, fluorescence spectroscopy, groundwater contamination.

1. Introduction

Our most valuable natural resource without which life would not be possible is water. It is essential for our survival and for our wellbeing. In this context, it is of utmost importance to have access to clean, uncontaminated water. Being used for agriculture, industries, human daily consumption and for a variety of household applications, ground water plays a major role in the ensuring an adequate livelihood for most of the Earth's inhabitants [1].

Water quality is influenced by the content of organic matter which can be determined by a number of allochtonous and autochthonous factors. Every natural aquatic system contains certain amounts of dissolved organic matter (DOM). Generally, DOM is comprised of a heterogeneous mixture of aromatic and aliphatic compounds, among which proteins, carbohydrates, lipids and humic substances are the most commonly detected [2]. However, its composition is not

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preset or predictable, being strongly influenced by the aquatic medium it populates and the surrounding environment [3]. It is important to study the content of DOM from aquatic media given the fact that its components have the ability of retaining and immobilizing organic and inorganic pollutants [4-7], thus increasing the risk of human contamination upon assimilation of polluted water.

In recent years, fluorescence spectroscopy has been used as a rapid and accurate technique for characterizing water samples [4,7-9]. It has the advantage of not requiring any type of reagent or sample preparation procedures [2]. When it comes to analyzing fluorescent components of aquatic organic matter, fluorescence spectroscopy has the ability of detecting them even at low concentrations, below 1 mg/L [2]. Of the multitude of DOM components, there are two main categories that possess fluorescent properties: proteins and humic substances [3]. On the one hand, the fluorescence property of proteins is determined by the presence of one or more of the three fluorescent amino-acids: tryptophan, tyrosine and phenylalanine, their specific signatures being directly related to the bacterial activity [3, 10-11]. On the other hand, the fluorescent signatures associated to humic substances are indicators of plant material decomposed due to biological and chemical processes occurring in the terrestrial and aquatic environments [12-15]. A body of water is considered to be clean and appropriate for human consumption if the humic and protein compounds are reduced and if there is a low ratio between the protein and humic substances markers [16].

The goal of this study is to evidence the DOM florescent signatures associated to proteins and humic substances and to present the benefits of using this technique for a preliminary evaluation of the water quality. Specifically, two fluorescence peak ratios will be used to identify and discriminate between pollution sources in groundwater from a rural area. This could create the premises of using fluorescence spectroscopy as an early warning system that could detect potential water contaminants.

2. Samples and methods

For this study, 14 water samples were collected from wells located in a rural area of Romania. The wells are situated in areas with different agricultural and landfill uses, having the potential of being contaminated with animal and human wastes. In order to obtain relevant results, each sample was analyzed within the first 24 hours from the collection.

To analyze the water quality using fluorescence spectroscopy, emission spectra can be acquired for different excitation wavelength, thus obtaining a matrix which represents a specific fingerprint characteristic for different aquatic system.

For the study of the fluorescence signatures of each sample, excitation-emission matrices (EEMs) were acquired using a FLS 920 spectrofluorimeter from Edinburgh Instruments equipped with a 450 W Xe lamp. For this purpose, emission spectra were registered in the 260-560 nm spectral range for excitation wavelengths ranging between 250 and 400 nm. The analysis of the EEM involved the identification of specific fluorescent fingerprints characteristic to proteins (amino acids) and humic substances. To this end, the nomenclature given by Coble [17] was used. Tryptophan and tyrosine amino acids, as well as humic substances, particularly humic acid, can be excited using a 260 nm wavelength. . The spectral domains characteristic to DOM compounds are highlighted in Figure 1. In Figure 1a, a detailed example of the fluorescent fingerprints is presented and their associated spectral domains that can be observed in an excitation–emission matrix, while in Figure 1b an emission spectrum is presented for excitation with 260 nm, where the spectral domains for the protein-like and humic-like signatures are highlighted.

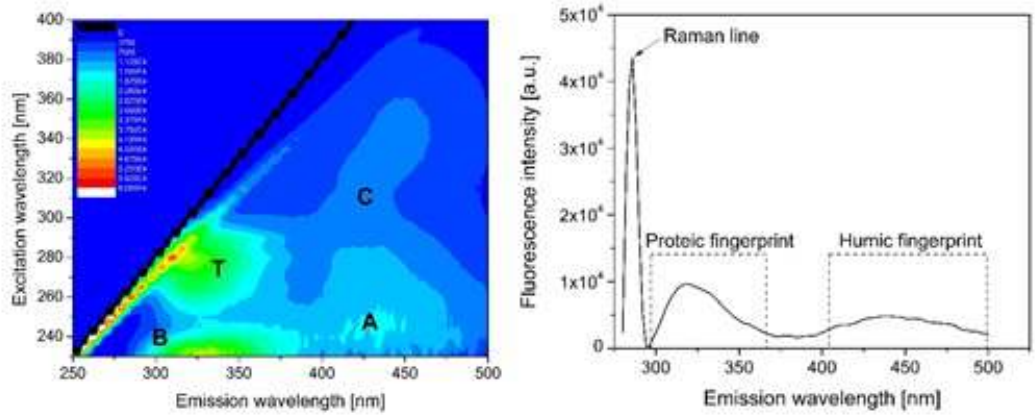

Fig. 1. Emission domains associated to the main DOM fluorophores:
a. Excitation-emission matrix; **b.** Emission spectrum.

As can be seen in Figure 1, the presence of the amino acid tryptophan (peak T) can be evidenced by a fluorescence maximum within the 320-350 nm emission domain. According to previous studies [12, 18], the presence of this particular maximum is characteristic to microbial activity. The second proteic maximum, which is associated to the amino acid tyrosine (peak B), presents a fluorescence maximum in the 300-310 nm range. With regard to the fluorescence fingerprints of humic substances, the humic acid exhibits a fluorescence emission maximum between 380 and 480 nm (peak A), while for the fulvic acids, the fluorescence maximum is registered in the 420-480 nm domain (peak C).

3. Results and discussions

In order to exhibit the specific fluorescent fingerprints of each of the 14 collected water samples, hereinafter referred to as S1-S14, excitation-emission matrices (EEM) were recorded. As an example, specific EEMs are illustrated in Figure 2 for S4, S6, S7 and S13 samples. It is important to mention that the areas situated in the vicinity of the water sources that provided the tested samples are very different and have a direct influence on the degree of contamination. The source of S4 sample has strong influences from domestic wastes, and presents areas of sediment accumulation. Another essential factor that influenced the quality of this water is the lack of connection to the sewer system in the area close to the well. It can be observed from Figure 2 that the EEM recorded for this particular sample exhibits strong fluorescent signals associated to peaks A and C, moderate intensities for peak T, while peak B has low fluorescent markers.

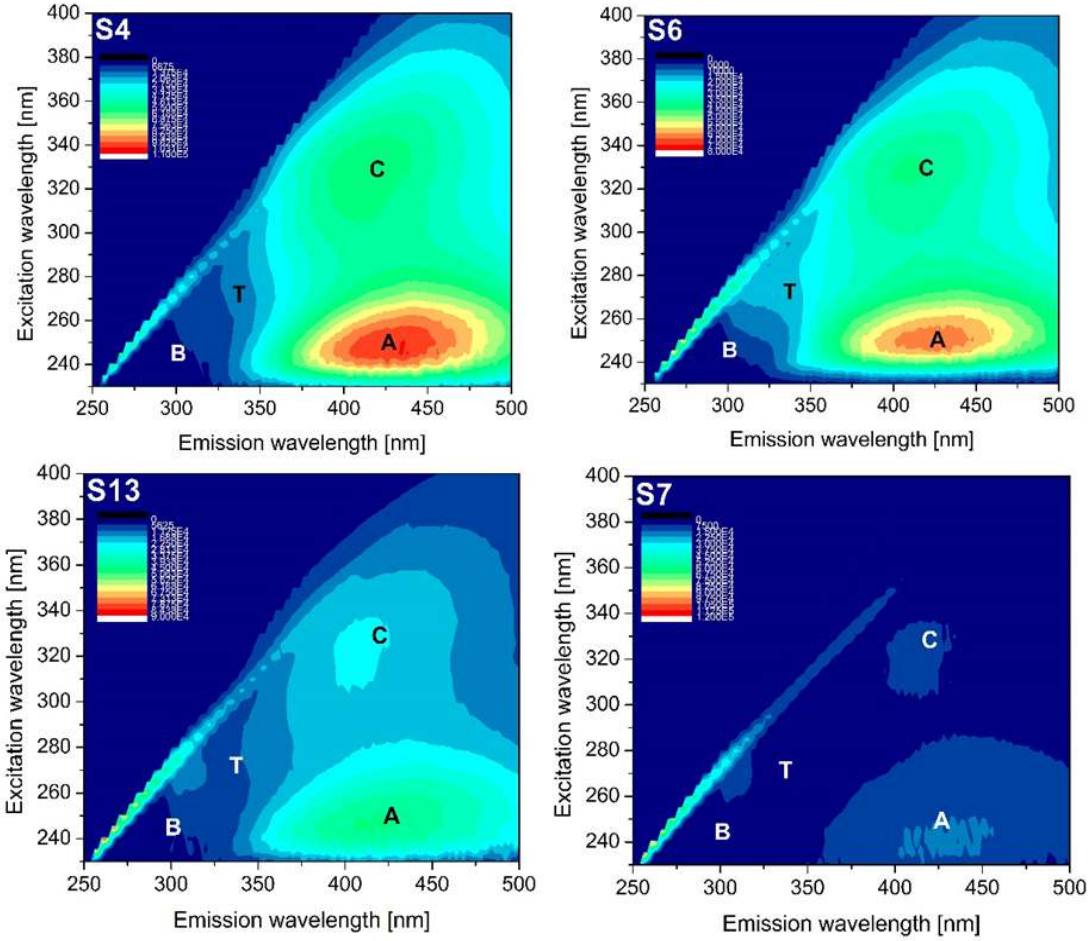

Fig. 2. Examples of excitation-emission matrices for the wells samples.

The EEM acquired for sample S6 presents pronounced signals associated to peaks A and C, although their intensities are slightly lower compared to those registered for sample S4. In this case, peaks T and B suggests a higher microbial contamination of this water source than the previous one analyzed. An explanation is that the water source of sample S6 has been subjected to soil infiltration caused by different excessive agricultural activities, domestic animal wastes as well as domestic contamination associated to household activities. For sample S13, both the proteic and humic fluorescent signatures appear to be more reduced. The source for this sample is located at relatively low altitudes and it is situated in the proximity of a river where household related wastes are being disposed. The last relevant EEM belongs to sample S7, which appears to contain the cleanest water from all the tested samples. It can be observed that in this case the humic fluorescent signature is not very intense and it is noticed only in the spectral region associated to peak A. There can also be observed a slightly pronounced fluorescent signal in the spectral region characteristic to proteins, which could be associated to peak B. This proteic fingerprint could be attributed to reduced soil influence from the lands located in the close vicinity of the source of sample S7. This sample was collected from an area that is relatively isolated from any possible waste contamination from animals or humans.

For evaluating the dominant DOM fraction in the analyzed samples, two ratios between relevant fluorescent maxima corresponding to humic and proteic components were determined. From the emission spectra acquired at an excitation wavelength of 260 nm, for each sample three values were isolated. The first one represents the fluorescence intensity of tyrosine (peak B) registered at an emission wavelength of approximately 305 nm, the second one is the maximum intensity associated to tryptophan (peak T) identified at around 315 nm and the third value represents the maximum intensity related to the presence of humic acids (peak A), which can be observed for an emission wavelength of about 445 nm. In this context, the T/A and B/T ratios are presented in Figure 3 and Figure 4 respectively.

The results obtained upon calculation of the T/A ratio provide useful information about the microbial content. Higher values suggest that the microbial fraction exceeds the quantity of humics, thus implying a possible microbial contamination of the water sample. The obtained results allowed us to classify the samples into three categories: samples with dominant humic fingerprint for values ranging from 0.1 to 0.5, those with moderate humic character having values in the 0.5-0.9 interval and samples with dominant microbial markers for values exceeding 0.9. It could be observed that most of the samples have a predominant humic character, with the exception of wells S8, S9, S12 and S13 situated in proximity of animal farms or in the vicinity of places where waste of household related activities are

disposed. These four wells have a dominant microbial DOM component, therefore they are not indicated for human consumption.

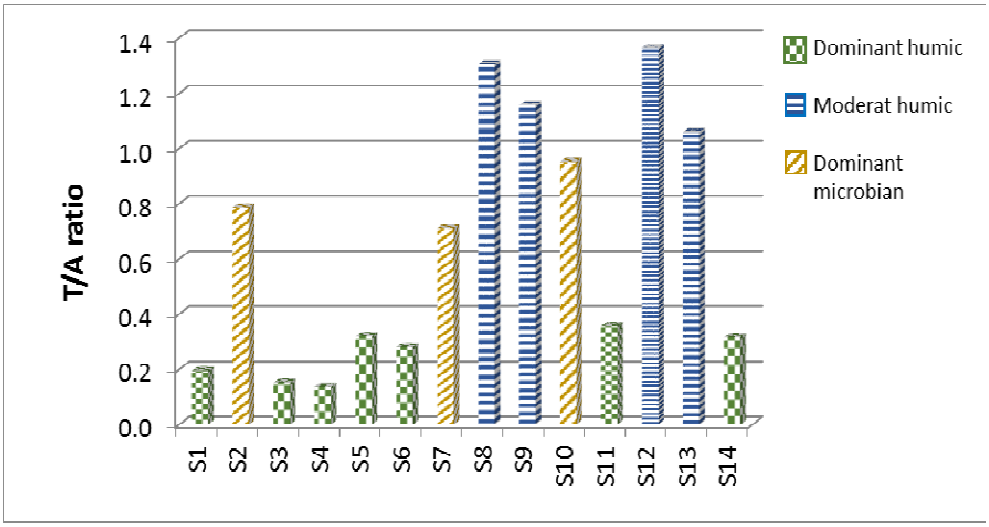

Fig. 3. T/A ratio indicator of microbial or humic content in water.

Further, in order to establish the nature of the microbial contamination source, the ratio between peaks B and T is relevant. A prominent fluorescent signature of tyrosine-like DOM component suggests that the source of the microbial contamination is most likely linked to animal wastes, while a lower value of the B/T ratio, thus meaning a more intense tryptophan-like fluorescent maximum, suggests that the source of the microbial contamination is comprised of human wastes [19].

Analyzing the data presented in Figure 4 referred to the B/T ratio, it can be observed that 9 out of 14 well samples have more intense peak B, compared with peak T, thus suggesting that these wells have a higher concentration of animal related microbial concentration, than wells S1, S2, S3, S6 and S10, where the predominant microbial signature is related to human waste. However, the samples with a tryptophan dominant fluorescent signature have B/T ratio values close to 1, suggesting that the contamination sources for these samples are almost in equal measure related to animal and human wastes. Overall, it can be observed that the majority of the samples presented a dominant tyrosine content, compared to the quantity of tryptophan component. This behavior is a little peculiar for water samples given the fact that in most cases the tyrosine characteristic signal is masked by the signal associated to tryptophan. This is due to the fact that tryptophan has a higher quantic efficiency than tyrosine, which in most cases transfers its energy to tryptophan [20].

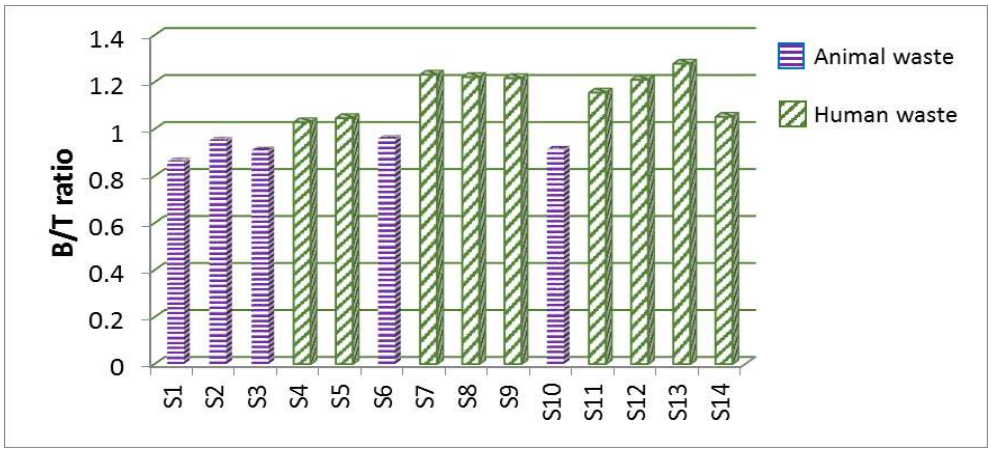

Fig. 4. Indicator of water contamination source.

Analyzing all the data acquired for the tested samples, it can be observed that in the case of the four particular samples for which detailed EEMs were presented, a clear image about the situation of the water sources occurs. For sample S4, the dominant humic component observed in the EEM from Figure 2 is supported by the results presented in Figure 3, the T/A ratio being 0.14. Furthermore, when it comes to determining the source of the proteic component from the sample, in Figure 4 can be noticed that for sample S4 the B/T ratio is slightly above 1, with a precise value of 1.03, which suggests that the source of the proteic component is from animal and human wastes, with a small predominance of the animal related signature. This conclusion is confirmed by the data related to the land use in the vicinity of the well, having strong influences from domestic wastes and animal wastes, considering that in most cases, each household possesses a number of animals on its property. Similarly, in case of sample S6, it can be observed that the proteic component is more pronounced than the humic one, compared to sample S4. According to Figure 4, the dominant proteic component is tryptophan, which suggests that the source is of human wastes disposed inadequately. In this case as well the data extracted from fluorescence spectroscopy are backed up by the environmental information regarding the vicinity of the well S6. Poor waste disposal registered in the area reveals the source of the water contamination. In the case of sample S13, the experimental results suggest that the proteic and humic signatures are almost equal, with a predominance of the tyrosine marker in the case of proteic component. These results are also correlated with the observations made locally in the vicinity of the well. Waste originating from the animals that reside near S13 is a direct contamination source. Last but not least, the fourth selected sample, S7, which appeared to be least contaminated, was collected from a well situated in a region far from houses and animal farms, thus explaining the fact that this sample in cleaner. Our findings suggest that this water

source has not been contaminated and there are still chances, in this case, to keep the contamination in check if the necessary measures are taken.

The novelty of this study consists in the classification of water samples upon the identification of the contaminant character using fluorescence ratios for emission spectra recorded at 260 nm excitation wavelength. This could create the premises of developing an early alert system that could identify and classify potential harmful contaminants present in water systems based on fluorescence spectroscopy.

Conclusions

The study highlighted the applicability of fluorescence spectroscopy as a reliable, fast and accurate method for characterizing water sources. To this end, samples were collected from underground water sources that supply wells used for human consumption and for various household activities from a rural area. The influence on the surrounding environment was established upon analyzing the fluorescence spectra registered for each of the 14 studied samples. In order to present a more clear view on the DOM components present in the water, excitation–emission matrices were presented, evidencing specific fluorescent fingerprints characteristic to clean, drinkable water and to contaminated water. Furthermore, in the case of the samples for which contamination was established, the type of organic matter input, either humic or proteic, was determined. To further detail our analysis, for the samples where proteic contamination was confirmed, the source of the contamination was also evidenced.

A thorough analysis of the T/A and B/T ratios obtained for only one excitation wavelength, helps scientists to identify more easily and quicker the type of water contaminant. The results can contribute to the development of a warning system concerning the water quality of different water sources.

Our findings suggest that environmental pollution should not be taken lightly, due to the fact that it can introduce contaminants to underground water sources, thus endangering the health of humans. Furthermore, the results we obtained prove that fluorescence spectroscopy can be successfully used as an initial step in detecting water sources contaminated with potential harmful compounds.

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