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LASER INDUCED FLUORESCENCE EFFICIENCY IN WATER QUALITY ASSESSMENT

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Abstract. Fluorescence data obtained by laser excitation at 266 nm were used for the first time to determine fluorescence properties of household and chicken farm discharges. Two fluorescence indices were applied: a modified humification index (HIX) and a new one, as tyrosine:tryptophan ratio (Tyr/Trp). HIX described very well the degree of contamination of the water system, while Tyr/Trp was useful in discriminating between the types of contaminants-household or chicken farms discharges. Results suggest that chicken farm wastes pollution could leave a specific fluorescence signature, when found in rivers, making possible their identification from the background fluorescence of the river waters.

Key words: laser-induced fluorescence spectroscopy, anthropogenic impact, water pollution, microbial index, tyrosine - tryptophan ratio.

1. INTRODUCTION

Laser has come a long way from its first appearance in the 1960s. Although it took several decades for the theoretical background to be put into practice, since then, lasers have developed greatly. They have found numerous applications, in fields such as science, medicine, entertainment, electronics, industry, military. In science, lasers can be used in the characterization of substances by laser induced fluorescence (LIF) spectroscopy, in many different areas, varying from chemistry [1,2] to DNA and genetic analysis [3,4], and even environmental quality assessment and monitoring [5,6].

The principle of LIF is based on inducing a fluorescent response in the investigated sample by illuminating it with a monochromatic, precise laser emission. The recorded spectrum represents a mixture of multiple individual fluorescent compounds that are present in the investigated sample. In the process of environmental monitoring, LIF can be applied to water studies due to the technique crucial advantages: lasers are versatile sources, have higher sensitivity and

selectivity compared to conventional spectrofluorometers. Using LIF, traces of fluorophores can be identified even from complex solutions like water samples [7].

Water quality can largely be characterized from the fluorescence signal of its dissolved organic matter (DOM) content. Fluorescent organic matter is an omnipresent constituent of the natural environment, derived from the decay of plant and animal matter, and is present in soil, river, marine and ground waters. DOM is composed of two fluorescent fractions: proteins (specifically amino acids, tyrosine and tryptophan) representing the universal marker for bacterial matter and humic substances (humic and fulvic acids) indicating the terrestrial input to the water system. DOM constituents can be found in different proportions and can describe the water quality based on their interactions with different external contaminants, reflecting the anthropogenic influence over the environment. Human daily activities can generate a wide range of organic contaminants, either from agricultural activity, accidental petroleum products leakage, household or animal farm discharges. In order to excite the two fluorescent fractions present in the water samples, UV lasers can be used [8].

Previous studies on farm wastes released in rivers [9-12] were performed using fluorescence spectroscopy in the form of emission-excitation matrices. To the authors' knowledge no research has been undertaken using LIF on household water and chicken farm discharges.

The aim of this study was to apply laser induced fluorescence spectroscopy for the evaluation of anthropogenic impact on river water quality. For this purpose a comparison of dissolved organic matter from different water sources was performed. LIF spectroscopy was used for the first time to determine fluorescence properties of household and chicken farm discharges. A 266 nm laser was chosen because its high energy allows the excitation of both microbial and humic-like fractions of the dissolved organic matter. Fluorescence indices were applied to identify the origin and degree of water contamination.

2. MATERIALS & METHODS

The LIF spectra were acquired using a YAG-Nd laser (266 nm) excitation source with 10 Hz frequency and 3-6 ns pulse duration, coupled with a Shamrock Spectrograph that recorded the spectra in the range of 280-600 nm with an intensified CCD camera from Andor. In order to avoid inner filter effects, absorption spectra were recorded with PerkinElmer LAMBDA 1050 spectrophotometer, in the 200-700 nm range. When absorption exceeded the value of 0.5, dilutions were performed and all data presented here were corrected for the corresponding dilution. Recorded data were processed using Microcal's ORIGIN 8.1 software.

Water samples were collected from five locations, with different known pollution sources. The first sampling point was on a clean river, which was used as control sample, the second collection point was on a river with household

discharges, the third one had an input from chicken farm wastes and the last two samples were collected from an urban river before and after a water treatment plant. Samples were measured within 24h after collection and stored in glass bottles at 4^0C before measuring.

3. RESULTS AND DISCUSSIONS

LIF spectra of water typically contain the inelastic scattering of laser emission on water molecules (Raman scattering signal). The Raman line is often used as an internal reference, making it a mean of standardisation for the fluorescence signal. Besides the inelastic scattering process, LIF spectra also contain fluorescence centres that are attributed to DOM, such as humic -like materials ranging between 400–500 nm emission wavelengths, and the fluorescent protein-like components (tyrosine and tryptophan) in the spectral domain 300–350 nm, as can be seen in Fig. 1. In general, the humic-like fraction exhibits less intense fluorescence compared to the protein-like, which is in most cases associated with anthropogenic impact. The protein-like constituent, tyrosine usually shows a fluorescence peak at around 305 nm, while tryptophan presents a larger fluorescence band between 320-355 nm. The emission wavelength of the peak varies, in this domain, depending on the surrounding medium and the tryptophan arrangement in the protein. Studies [13] have shown that tryptophan embedded in the protein fluoresces between 320–340 nm, while tryptophan residues which are directly exposed to water emit fluorescence between 340–355 nm. Therefore, we can assume that the large band belonging to tryptophan is given by the presence of both embedded and unembedded residues. Lasers high energy may be responsible for the fact that discrimination among types of tryptophan and amino acids can be done. Because the two amino acids have closely positioned optical spaces and tyrosine fluorescence has lower quantum efficiency, this amino acid is more susceptible to energy transfer to tryptophan.

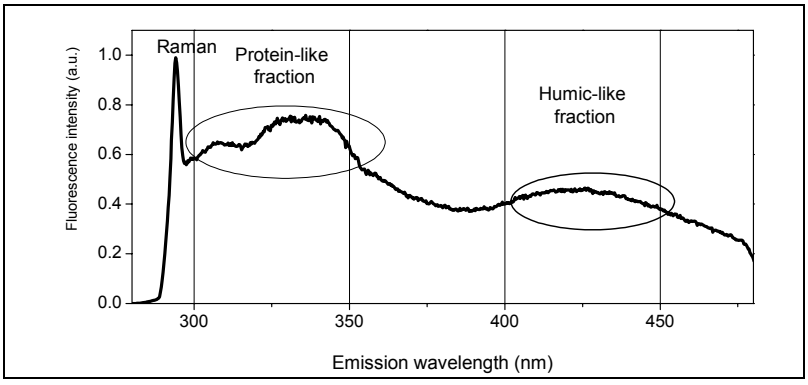


Fig. 1 – LIF spectrum with illustration of protein-like and humic-like fluorescence centres.

Figure 2 presents the LIF spectra for distilled water and clean river water. As expected, the spectrum of distilled water has a well defined Raman line and no other fluorophores centres. The clean river water sample also shows a well intense Raman signal, but apart from that peak, a humic-like component (400-500 nm emission wavelengths) is present, which is typically observed at uncontaminated river water.

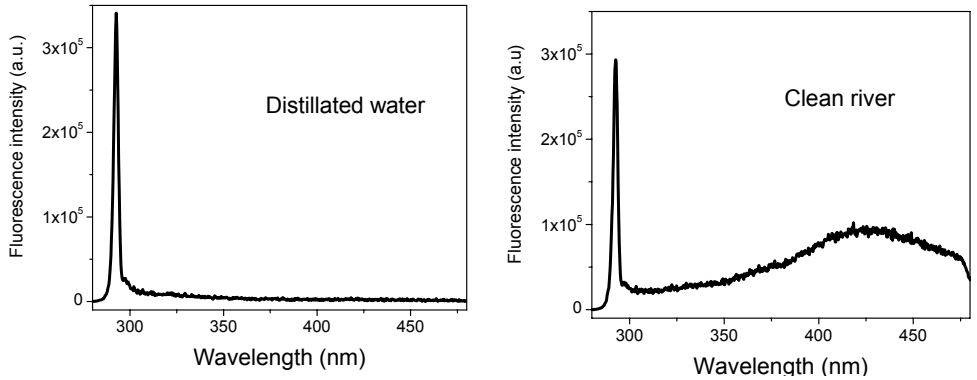
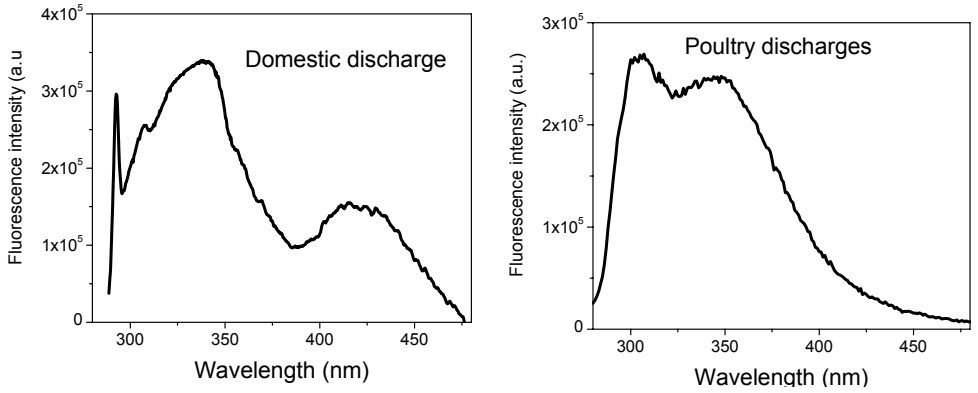


Fig. 2 – LIF spectra for distilled water and clean river water.

In order to determine the effectiveness of LIF technique, samples collected from two highly contaminated rivers were analysed: river with household sewage impact and river containing animal waste, specifically poultry. The LIF spectra for these samples are presented in Fig. 3. The sample collected from a river with domestic discharges input shows two protein-like fractions, tyrosine (300–309 nm) and tryptophan (323–350 nm); also, the humic-like component can be observed with a maximum around 423 nm (Fig. 3a). The high protein-like fluorescence intensity indicates the presence of human sewage waste [14]. This contamination is due to the lack of a treatment station, the domestic wastes being discharged directly into the river, which leads to a very complex matrix of components. It must be emphasised that, the tryptophan-like component exhibits significantly higher fluorescence intensity then tyrosine. Again, tryptophan shows a large band, which may be attributed to both types of tryptophan, embedded and unembedded. As for the humic-like fraction, it can be noticed that, compared to the clean river (Fig. 2), it tends to present a blue shifted maximum which indicates, as suggested by Wu et al. [15], a decrease in aromaticity.

The sample collected from the river with chicken farm wastes input also exhibits a significant microbial fraction, but less intense contribution from humic-like component (Fig. 3b). The differences between human and chicken waste discharges are highlighted by the protein-like fluorescence. The fluorescence spectrum of the poultry sample reveals high intensities for both amino acids, but in particular, tyrosine has shown higher values compared to tryptophan. These

findings are contrasting to those obtained for domestic discharge samples, which suggests that farm waste pollution could leave a mark on river waters due to their distinctively high tyrosine fluorescence signal.



a) b)

Fig. 3 – LIF spectra for polluted rivers:
a) household discharges impacted river and b) chicken farm wastes impacted river.

Aiming to evidence the LIF capacity to determine DOM, water samples collected before and after the treatment station were tested. Previous studies using fluorescence spectroscopy, in the form of emission-excitation matrix (EEM), have demonstrated that DOM from untreated sewage samples is characterized by high levels of protein-like and humic-like fluorescence [11, 12]. As illustration, specific fluorescence spectra can be seen in Fig. 4 where LIF recordings of river water before and after a water treatment plant are shown.

The sample collected before the treatment plant showed high microbial activity, represented by a fluorescence band in the 315-385 nm wavelength domain and also important humic-like fraction in the emission spectral range of 420–480 nm. After the purification station, it can be seen that the microbial component has been almost completely removed and the Raman line can be observed. The humic substances content has also been reduced, but not as much as the protein fraction. It can also be pointed out, that the humic-like peak is red shifted, compared to clean river sample (Fig. 2), which indicates the presence of more hydrophobic fractions.

As can be noticed from the presented spectra, the position and the intensity of fluorescence peaks may vary depending on organic matter source (e.g. site geography, natural contributions or external contaminants-agriculture runoff, household or farm discharges and tryptophan state that can be folded or unfolded in the protein [12]). Hence, by determining the ratio between different fluorescence peaks, it may be possible to quantify the degree and nature of water contamination. Previous studies have shown that discrimination can be made between the organic matter's sources by using various fluorescence indices [16, 17].

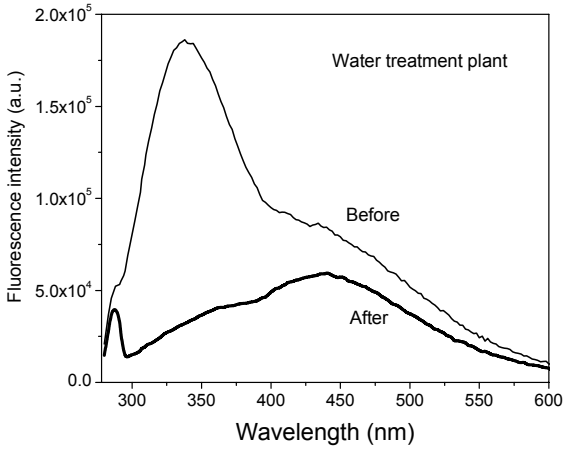


Fig. 4 – LIF spectra before and after water treatment plant.

For this experiment, the humification index (HIX) [16], was chosen in order to evaluate the balance between humic matter and microbial content. In order to determine the degree of microbial contamination we adjusted HIX, so as to best encompass the spectral domains in which the microbial and humic –like fractions appear in our samples, at excitation wavelength of 266 nm. Thus, the humification index was determined as the ratio of fluorescence intensity between 300-350 nm and 410-460 nm. Using HIX we obtained a value of 0.3 for clean water and values higher than 2 for sewage impacted rivers in agreement with other results from literature. For the first time we determined a HIX value of 8.83 for poultry water. The effectiveness of the water treatment processes was evaluated by comparing the HIX values of samples collected before and after the treatment plant. A decrease from 2.1 to 0.47 of HIX values denotes a good cleaning efficiency of the water treatment plant.

Taking into account the detected difference in the fluorescence intensity of protein-like components (tyrosine and tryptophan) found in the spectrum of chicken farm wastes in comparison with domestic wastes, we propose a new fluorescence index as the ratio of these two components, hereafter named Tyr/Trp ratio. This ratio has been calculated using $\text{Tyr/Trp} = \frac{Area(F_{300:309})}{Area(F_{334:343})}$.

A value higher that 1 (1.09) was obtained for the samples originated for the river water with chicken wastes input while a value lower than 1 (0.7) was obtained for the river water contaminated with domestic discharges.

The high ~ 1:1 intensity ratio of the two fluorescence centres (tyrosine and tryptophan) obtained from river with chicken farm waste input has not been previously observed in the natural environment, except for pig and cattle slurry where a value close to 1 was reported [11]. The different ratios between tyrosine

and tryptophan fluorescence intensities for the two polluted water samples (household and farm discharges) can be attributed to the specific contents of the wastes input. This demonstrates that the new fluorescence index Tyr/Trp is useful in discriminating between the types of water contaminants: household or chicken farms discharges.

The ability of laser induced fluorescence spectroscopy to differentiate between the pollution sources and to evaluate the degree of water microbial contamination represents a useful and fast technique in the field of aquatic ecology. The 266 nm excitation wavelength can be incorporated in portable spectrometers allowing the on-site application to characterize and identify the different pollution sources within surface water subjected to a range of external influences.

4. CONCLUSIONS

Laser induced fluorescence spectroscopy data were used for the first time to determine the fluorescence properties of river waters with domestic and poultry discharges. LIF seems to be very efficient in the detection of the relevant fluorophores, allowing to evidence, besides the humic-like fraction, both microbial components (tyrosine and tryptophan) of DOM.

The water quality was determined with the aid of two fluorescence indices: the modified HIX and a new introduced index, the Tyr/Trp ratio. HIX indicated the degree of water contamination, with values ranging from 0.3 for clean river water, around 2 for human waste impacted river and more than 8 for the chicken waste impacted river. The Tyr/Trp ratio was useful in discriminating between the types of wastewater contamination: household or chicken farms discharges. Results showed that chicken farm discharges have a particularly intense Tyr/Trp ratio, with a specific signature in waters. Further application of the LIF technique to water pollution studies is required to verify the utility of new introduced fluorescence index.

LIF analysis of human and chicken waste impacted rivers can lead to a quick detection method, for *in-situ* water quality monitoring. Such method could indicate the appropriate way of water cleaning, taking into account the various levels and sources of contamination, thus improving the overall environmental quality.

REFERENCES

1. C.R. Warren, *Rapid and sensitive quantification of amino acids in soil extracts by capillary electrophoresis with laser-induced fluorescence***,** Soil Biol. Biochem., **40**, *4*, 916–923 (2008).
2. S. Laville, C. Goueguel, H. Loudyi, F. Vidal, M. Chaker, M. Sabsabi, *Laser-induced fluorescence detection of lead atoms in a laser-induced plasma: An experimental analytical optimization study*, Spectrochim. Acta B**, 64**, *4*, 347–353 (2009).

3. J. Ren, N. Fang, D. Wu, *Inverse-flow derivatization for capillary electrophoresis of DNA fragments with laser-induced fluorescence detection*, Anal. Chim. Acta., **470**, *2*, 129–135 (2002).
4. W. Wang, L. Zhou, S. Wang, Z. Luo, Z. Hu, *Rapid and simple determination of adenine and guanine in DNA extract by micellar electrokinetic chromatography with indirect laser-induced fluorescence detection*, Talanta**, 74**, *4*, 1050–1055 (2008).
5. M. L. Pascu, N. Moise, A. Staicu, *Tunable dye laser applications in environment pollution monitoring*, J. Mol. Struct.**, 598**, *1*, 57–64 (2001).
6. M. González-Pérez, D.M.B.P. Milori, L.A. Colnago, L. Martin-Neto, W.J. Melo, *A laser-induced fluorescence spectroscopic study of organic matter in a Brazilian Oxisol under different tillage systems*, Geoderma, **138**, 20–24 (2007).
7. C. L. Stevenson, T. Vo-Dinh, *Analysis of polynuclear aromatic compounds using laser-excited synchronous fluorescence*, Anal. Chim. Acta, **303**, 247–253 (1995).
8. V. I. Fedorov, *Studying Organic Species in Water by Laser Fluorescence Spectroscopy with a Source of Excitation in Mid-UV Range (266 nm)*, Water Resour., **32**, *5*, 549–554 (2005).
9. P. Westerhoff, W. Chen, M. Esparza, *Fluorescence analysis of a standard fulvic acid and tertiary treated wastewater*, J. Environ. Qual., **30**, 2037–2046 (2001).
10. D.M. Reynolds, *Rapid and direct determination of tryptophan in water using synchronous fluorescence spectroscopy*, Water Res., **37**, 3055–3060 (2003).
11. A. Baker, *Fluorescence properties of some farm wastes: Implications for water quality monitoring*, Water Res., **36**, 189–194 (2002).
12. A. Baker, R. Inverarity, *Protein-like fluorescence intensity as a possible tool for determining river water quality*, Hydrol. Process.**, 18**, 2927–2945 (2004).
13. R.W. Alston, L. Urbanikova, J. Sevcik, M. Lasagna, G.D. Reinhart, J.M. Scholtz, C.N. Pace, *Contribution of Single Tryptophan Residues to the Fluorescence and Stability of Ribonuclease Sa*, Biophys. J.**, 87**, 4036–4047 (2004).
14. E. Pfeiffer, G. Pavelescu, A. Baker, C. Roman, C. Ioja, D. Savastru, *Pollution analysis on the Arges River using fluorescence spectroscopy*, J. Optoelectron. Adv. M., **10**, *6*, 1489–1494 (2008).
15. F. C. Wu, R.D. Evans, P.J. Dillon, *Separation and characterization of NOM by high-performance liquid chromatography and on-line three-dimensional excitation emission matrix fluorescence detection*, Environ. Sci. Technol.**, 37**, *16*, 3687–3693 (2003).
16. A. Zsolnay, *Dissolved organic matter: artefacts, definitions, and functions*, Geoderma**, 113**, 187–209 (2003).
17. E. M. Carstea, L. Ghervase, G. Pavelescu, D. Savastru, *Assessment of the anthropogenic impact on water systems by fluorescence spectroscopy*, Environ. Eng. Manag. J.**, 8**, *6*, 1321–1326 (2009).