JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS Vol. 10, No. 6, June 2008, p. 1489 - 1494

Pollution analysis on the Arges River using fluorescence spectroscopy

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Fluorescence spectroscopy was used to evaluate the influence of untreated wastewaters on Arges river water quality. Samples collected from the Lower Basin of the Arges River were analyzed using laser induced fluorescence spectroscopy (LIFS) and excitation-emission matrix (EEM) fluorescence. Specific fluorescence fingerprints for Arges River water were shown. Using LIFS the influence of the wastewater discharged from urban areas and nearby farms was shown by protein-like fluorescence presence (tyrosine-like and tryptophan-like). The decrease in pollution downstream due to auto purification process (dilution and breakdown of the organic fraction) was also shown. River protein-like fluorescence intensity determined by LIF correlated strongly with fecal coliform content, measured with standard biological method. This proved that laser induced fluorescence can be a rapid and efficient tool in detecting urban sewage contamination, taking into account that LIFS is more sensitive method for picking up weaker fluorescence signals, such as that from tyrosine and the presence of folded and unfolded tryptophan residues.

(Received May 4, 2008, after revision May 28, 2008; accepted June 4, 2008)

Keywords: Fluorescence spectroscopy, Water pollution, Laser induced fluorescence

1. Introduction

The Lower Basin of the Arges River represents one of the most polluted areas in Romania due to the concentration of the industrial, agricultural and household degradation sources carried along the river, especially from Bucharest metropolitan area (2.5 million inhabitants). These municipal wastes are directly discharged into the river with no wastewater treatment station [1], [2].

Numerous studies have proposed the fluorescence technique to be used in water quality studies [3-5]. It offers real time data and can be used as a surrogate for water quality tests, like biochemical oxygen demand (BOD) or chemical oxygen demand (COD), which either require a large number of days for analysis, or have a high cost per sample [3, 6].

The fluorescence in natural waters is attributed mostly to humic substances derived from the breakdown of plant material [7] and proteins produced through microbial activity [6, 8]. The fluorophores denoting the presence of proteins in water are tryptophan and tyrosine. The fluorescence intensity of these compounds can be used as indicators of water quality and can establish the specific fingerprint of water sources. For instance, the fluorescence of natural river waters is dominated by humic substances, but in the case of sewage waste [9], farm waste [4] or landfill leachates [10], tryptophan and tyrosine present a more intense fluorescence than humic substances. Among the spectroscopic techniques, LIFS has several advantages, such as narrow bandwidth excitation, high energy and short pulse excitation. LIFS selectively and efficiently excites the fluorophores in organic matter. For these reasons, LIFS techniques have found many applications in water quality monitoring and pollution detection.

The paper aims to determine the water quality in the Lower Basin of the Arges River where it is affected by domestic, industrial and farm wastewaters, in order to compare the fluorescence characteristics with fundamental geochemical water quality. To obtain the specific fluorescence fingerprint, two fluorescence techniques have been applied: laser induced fluorescence (LIF) and excitation-emission matrix (EEM). Physical, chemical and biological parameters of the water have also been determined.

2. Catchments and methods

During the period from June 2006 to February 2007 river water samples have been collected from six different locations (fig.1): 1 – *Sabar* on Sabar River, 2 – *Colibasi* on Arges River, 3 - *Hotarele* downstream of Sabar river confluence, 4 - *Budesti* on Dambovita River that crosses Bucharest, 5 – *Soldanu* downstream of Dambovita river confluence and 6 - *Clatesti* on Arges River before the Danube confluence. In order to show the urban impact over the river water quality only three of them are considered critical study points: Hotarele - before the

confluence with Dambovita River, Budesti - at the confluence and Clatesti – after the confluence.

The other three samples, Sabar, Soldanu and Colibasi, are used to provide additional sites to investigate the relationship between river water fluorescence and the different anthropogenic sources of contamination to the Arges River. The collected samples were filtered (0.8 μm pore size filter) and stored 24 h at 5 ^{o}C degrees before measurements were made.

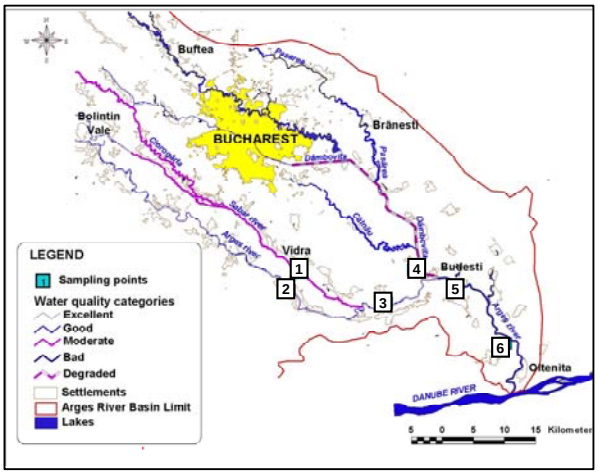


Fig. 1. Sampling points on Arges River and tributaries.

For laser induced fluorescence, a Shamrock Spectrograph and ICCD Andor were used. YAG-Nd laser was used (266 nm, 10 Hz frequency, 3-6 ns pulse duration) as excitation source.

Fluorescence measurements were also performed with Varian Cary Eclipse Spectrofluorimeter. To produce fluorescence excitation–emission matrices (EEMs) excitation wavelengths (Ex) were incremented from 200 to 420 nm at 5 nm intervals; for each excitation wavelength the emission (Em) was detected from 280 to 500 nm at 0.5 nm intervals, using previous methods [4].

Physical (temperature, pH, conductivity, turbidity and transparency) and chemical (organic indicators, salinity, toxic indicators) parameters were determined using standard methods.

3. Results and discussion

3.1 Physical and chemical parameters

Physical and chemical parameters have been determined in order to establish the degree of contamination, as shown in Table 1. *The water temperature* varied between 11.2 ^{o}C in February and 25.4 ^{0}C in June (an average of 7.6-8 ^{o}C), the highest values being registered at Budesti site (+0.5 – 4 ^{0}C higher compared with the other sampling points) and the lowest ones at Colibasi and Sabar. The increase of temperature is releted to the releases of wastewater generated in Bucharest, with higher values of temperatures determined by the domestic sources, but also by industrial units (mainly energetic, metallurgical, machine construction and food production branches). Temperature increasing has effects on the physical-chemical processes (mainly oxygen transfer between phases) taking place in the aquatic ecosystems.

Water transparency shows low values (2-5 cm) due to the high concentration of suspended matter. Turbidity has a high level in Budesti section (maximum 64.2 FTU), due to the organic and inorganic particle suspentions.

Conductivity presents higher values for Budesti sample as it is more turbid then the other samples. As indicators of the organic load we have considered dissolved oxygen (DO), chemical oxygen demand (COD) and biochemical oxygen demand (BOD). The lowest concentration of oxygen has been registered for Budesti site, where during the summer appear frequent cases in which the concentration is close to 0 mg/l (Table 1).

Budesti samples contained high quantities of nitrogen, chlorides, iron, cadmium and lead. Insignificant values of detergents, pesticides and polyaromatic hydrocarbons have been detected at all samples [2]. These results prove that the only significant influence to the Arges water quality is given by urban wastewater discharges.

Table 1. Physical and chemical parameters in Arges Lower Basin between 2006-2007.

Water samples from sites shown in fig.1.	Temp. (^{0}C)	Turbidity (FTU)	pH	DO (mg/l)	Conductivity (μS)	BOD (mg O_2/l)	COD (mg O_2/l)
Campaign I (June 2006)							
Sabar	21.4	41.3	7.75	12.5	454	55	100
Colibasi	23.5	38.6	8.2	13.2	259	48	81
Hotarele	24.8	37.9	8.1	12.7	296	43	75
Budesti	25.0	52.3	7.5	0.3	450	95	167
Soldanu	25.4	39.2	7.4	7.8	292	80	143
Clatesti	26.0	44.8	7.76	7.3	356	60	110

Water samples from sites shown in fig.1.	Temp. (^{0}C)	Turbidity (FTU)	pH	DO (mg/l)	Conductivity (μS)	BOD (mg O_2/l)	COD (mg O_2/l)
Campaign II (October 2006)							
Sabar	14.9	31.7	7.66	7.4	522	59	106
Colibasi	17.6	28.7	7.83	8.8	318	61	110
Hotarele	17	23.2	8.01	8.6	578	54	97
Budesti	19	59.9	7.64	0.2	676	145	214
Soldanu	17.4	45.3	7.62	0.3	569	76	133
Clatesti	18.4	41.1	7.65	3.0	540	77	261
Campaign III (February 2007)							
Sabar	10.1	44.1	7.71	6.1	541	58	98
Colibasi	11.2	31.2	7.91	8.1	312	53	90
Hotarele	10.9	33.6	8.24	6.5	715	48	82
Budesti	14.1	64.2	7.72	0.2	738	120	132
Soldanu	11.4	48.2	7.69	0.6	725	62	207
Clatesti	13.3	43.1	7.84	1.5	683	59	100

3.2 Emission excitation matrix fluorescence

Fluorescence excitation-emission matrices recorded for water sampled at Hotarele, Budesti and Clatesti (fig. 2 a, b, c) show the position of the principal fluorophores that are typically observed in rivers and wastewaters [3 - 7]. The fluorescence spectrum for Hotarele (fig. 2a) is dominated by the natural contribution of humic substances (excitation wavelength between 220 - 250 nm and emission wavelength in the range 360 - 450 nm). Relatively low intensity tryptophan-like fluorescence maximum at 350 nm with excitation wavelength at 232 nm comes more likely from farm waste products. No fluorescence from tyrosine is present in the spectrum.

A change in water composition at Budesti point is clearly shown in its fluorescence spectrum (fig. 2b). All peaks (A, C, B and T as named by Coble [7]) characteristic for untreated wastewaters water are present in EEM. Unlike Hotarele, Budesti sample emits high intensity fluorescence at excitation 228 nm and emission 280-305 nm (peak B), maximum that is attributed to tyrosine [11, 12]. A highly intense tryptophan-like fluorescence (peak T: excitation at 220 – 230 nm and emission in the range 325 - 375 nm) and humic substances (peak C: excitation at 320 – 340 nm and emission at 400 – 440 nm) can also be seen in the spectrum.

Another excitation/emission pair can be seen for humic substances at Ex/Em = 340 nm / 420 nm and for tryptophan at Ex/Em = 289 nm/ 340-370 nm. The direct discharge of sewage waste from Bucharest into the river is confirmed by the intense protein-like fluorescence (B and T peaks). This indicates intense microbial activity that can disrupt the stability of the aquatic ecosystem and affect the human health.

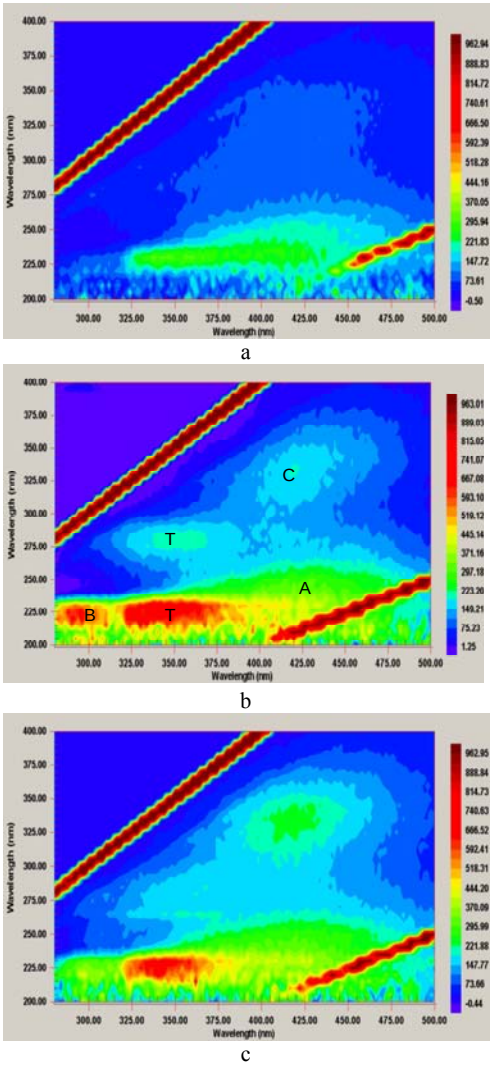


Fig. 2. Excitation-emission matrices from the sample sites a) Hotarele (site 3), b) Budesti (site 4), c) Clatesti (site 6).

Due to the auto purification process (dilution and breakdown of the organic fraction) the degree of microbial contamination is reduced along the river until Clatesti (about 52 km from discharge point), evidenced by the absence of tyrosine-like fluorescence and a less intense protein-like fluorescence maxima (Fig. 2c) present in the spectrum compared to Budesti.

3.3 Laser induced fluorescence

The laser induced fluorescence method has also been applied to the samples in order to obtain more information about water pollutants, taking into account its ability to excite molecules even with low quantum efficiency. An example of fluorescence spectra at 266 nm excitation wavelength is shown in Fig. 3 for Budesti (site 4), measurements made 2 and 50 days after sampling, respectively. The spectra reveal the water Raman band at 294 nm, a relatively intense broad fluorescence band with a maximum at around 330 nm and a relatively less intense band centered on 425 nm. The band centered at 330 nm that does not appear in the EEM, can be decomposed into three maxima corresponding to 305 nm, 324 nm and 358 nm. This band disappeared after 50 days.

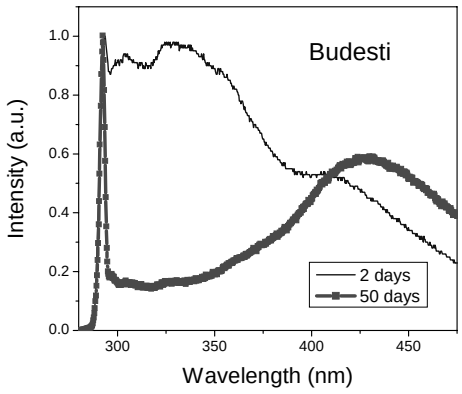


Fig. 3. LIF spectra for water sampled from Budesti site

Tyrosine-like fluorescence is relatively difficult to see in EEM fluorescence spectra, given its relatively low fluorescence efficiency when compared to tryptophan, due to interference caused by its close location to the tryptophan fluorescence centre, energy transfer between tyrosine and tryptophan fluorophores and the interference of the Raman line of water. At laser excitation (fig.3) the tyrosine-like fluorescence can be seen at 305 nm beside the tryptophan-like fluorescence at 358 nm. Some sources [13, 14] indicate that tryptophan residues directly exposed to water exhibit fluorescence at about 340–350 nm, while folded tryptophan residues fluoresce at lower wavelength. Due to the lasers' high energy it may be possible to also obtain intense fluorescence from the folded tryptophan, despite its low quantum efficiency. Therefore, we hypothesise that the fluorescence peak at 324 nm belongs to tryptophan residues folded into proteins (e.g. living or dead cellular material) and the peak at 358 nm is exhibited by free tryptophan residues (e.g. exudates and lysis material) dissolved in the water. In consequence, we hypothesise that LIF might be used to provide useful information on the microbial nature of protein-like fluorescence.

The anthropogenic influence on LIF spectra is shown by comparing the protein-like fluorescence (330 nm) for all samples. Figure 5 illustrates almost no influence from the environment on Colibasi, Sabar and Hotarele samples (sites 1-3) in comparison with Budesti sample (site 4), which is highly contaminated with sewage waste. Gradually, due to selfpurification (especially dilution) the contamination decreases (Soldanu, Clatesti) as the river flows to its downstream.

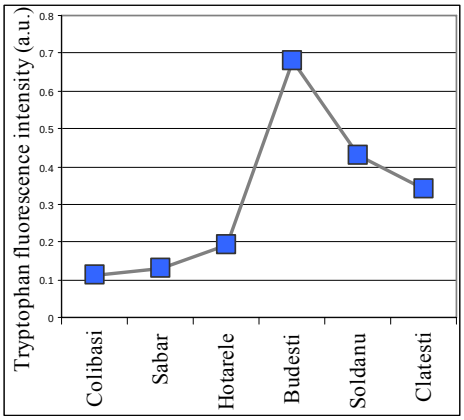


Fig. 5. The autopurification process for Arges River protein-like fluorescence has been taken at λ_{em} 330 nm.

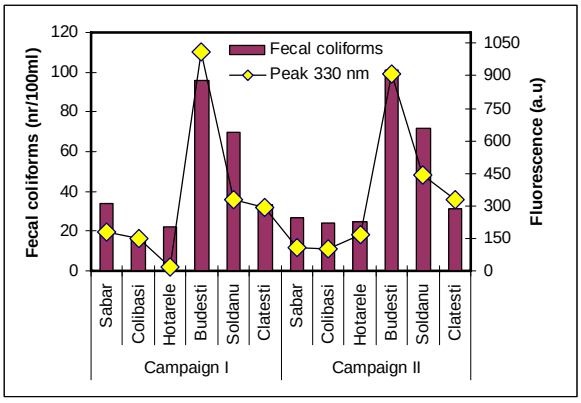
3.4 LIF correlation with fecal coliform

It has been well documented [9, 10] that protein-like fluorescence intensity corresponds to the presence of bacteria in the water samples. A conventional indicator of bacteria presence is given by the fecal coliform content. Significant quantities of fecal coliforms are found at Budesti, the source of which is coming mostly from waste discharges from Bucharest. Fecal coliform bacteria can enter rivers through direct discharge of waste from mammals and birds, from agricultural practices and from untreated human sewage. Fecal coliform analysis through standard methods takes ~2 days. One goal has been to determine whether protein-like fluorescence can be used as a surrogate to detect in real time the presence of fecal coliform bacteria. In order to validate the results, two different campaigns (June and October) have been used for comparison.

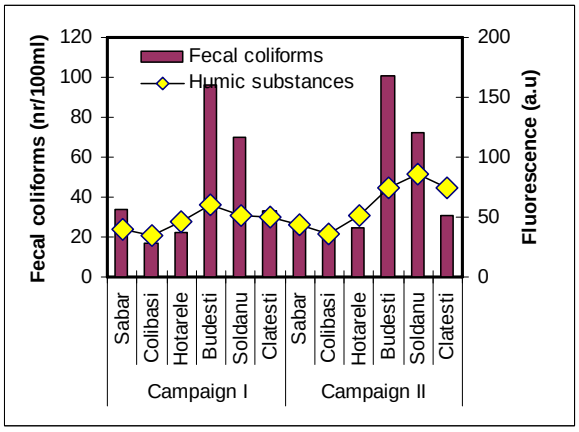
As can be seen in Fig.6a, a good correlation between the fluorescence intensity at 330 nm emission wavelength (laser excitation at 266 nm) and fecal coliform content has been obtained. The correlation factor lies between 0.9 - 0.95 for the two campaigns. The Budesti samples exhibit the highest values for both protein-like fluorescence

intensity and fecal coliform content. Contrasting results were obtained for samples taken from Sabar, Colibasi and Hotarele. The low values show only a small contribution of fecal coliforms belonging agricultural activities such as spreading manure or animal wastes being washed into the rivers during rain events.

As it is expected a low correlation (0.71 correlation factor) was obtained between the fluorescence intensity of humic substances (E_x/E_m= 266 / 430 nm) and fecal coliform content (Fig. 6b). This lack of correlation is explained by the plant matter and soil origin of the humic substances.



a



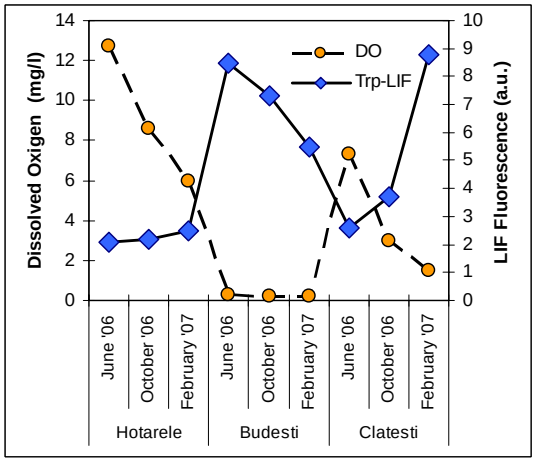
b

Fig. 6. Correlation between the content of fecal coliforms in water and fluorescence intensity of a) protein-like, b) humic substances.

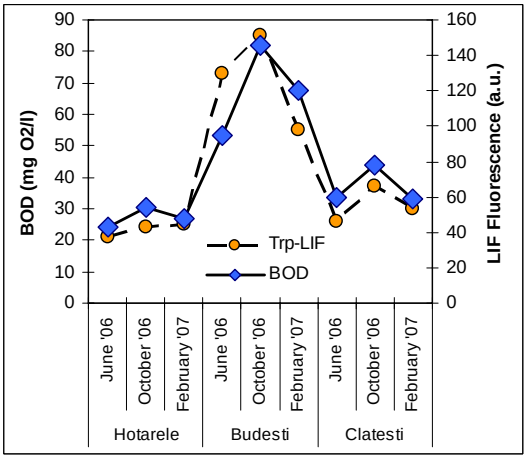
From the graph it is quite obvious that the most important contribution to water contamination by fecal coliforms comes from human sewage, as obtained for Budesti sample. Fecal coliform content decreases continuously with increasing distance from Budesti, at Soldanu and Clatesti points, due to self purification process.

3.5 LIF correlation with physical and chemical parameters

LIF measurements have shown good signal even some sites are turbid and ph varied between 7.4 and 8.1. The tryptophan intensity (358 nm) has been correlated with dissolved oxygen (DO) and biochemical oxygen demand (BOD) for the samples: Hotarele, Budesti and Clatesti. Samples have been collected and measured in different seasons (June, October, and February) in order to eliminate the temperature influence. As can be seen in figure 7, tryptophan fluorescence intensity is inversely proportional to dissolved oxygen and proportional with the values for BOD (correlation factor 0.92).



a



b

Fig.7. Correlation between tryptophan and a) dissolved oxygen, b) biochemical oxygen demand

4. Conclusions

The influence of untreated domestic sewage on river water quality was shown by fluorescence spectroscopy. EEM and laser induced fluorescence methods have been employed in order to detect the principal contaminants at

samples taken from Arges River and its tributaries which receive strong anthropogenic inputs.

Using laser induced fluorescence we hypothesise that more detailed information can be obtained on the evolution of tyrosine and the presence of folded and unfolded tryptophan residues into the wastewater. Fluorescence intensity strongly correlates with fecal coliform content which proves that laser induced fluorescence can be a rapid and efficient tool in detecting urban sewage contamination.

The results showed the significant impact of urban sewage discharges on the river water quality. This urges the need to develop wastewater treatment facilities at the discharge points and start the processes of ecosystem reconstruction.

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