

*Dedicated to Professor Valentin I. Vlad's 70<sup>th</sup> Anniversary*

## SPECTRAL FINGERPRINTS OF GROUNDWATER ORGANIC MATTER IN RURAL AREAS

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*Abstract.* Three dimensional fluorescence spectroscopy, as a spectral fingerprint of dissolved organic matter was used to examine the impact of surrounding areas on groundwater sources (wells) situated in a rural village, Vartoapele de Jos, Teleorman County. Fluorescence indices allowed discriminating between water types in connection with the proximity of different water contamination sources.

*Key words:* fluorescence spectroscopy, dissolved organic matter, groundwater pollution, fluorescence indices

### 1. INTRODUCTION

Groundwater from private water wells is the major source of drinking water in rural and peri-urban areas from Romania. The current problems facing groundwater quality derive from anthropogenic activities: nitrate and pesticides contamination emanates from fertilizer application to agricultural lands and biochemical reactions of untreated human and animal wastes storage [1–4].

Water quality can be characterized using the fluorescence signal of its dissolved organic matter (DOM) components: proteins (specifically the amino acids, tyrosine and tryptophan), which represent the universal marker for bacterial matter, and humic substances (humic and fulvic acids), which indicate the terrestrial input to the water system [5, 6]. 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 [7–10]. Using fluorescence spectroscopy in the form of excitation-emission matrices (EEM), a 3D map can be obtained, that symbolizes a fingerprint specific to different fluorophores, being a spectral signature, unique for a given

mixture. The topography of EEM (the number of fluorescent maxima, their coordinates ( $\lambda_{ex}$ ) on the plot, the shape of contour lines of equal intensity on the plot) allows identification of organic compounds in water and estimation of their concentration.

In this study, three-dimensional fluorescence spectroscopy was used to evidence the fluorescence spectral fingerprint of DOM and identify the specific features for some wells situated in a rural area. Fluorescence indices, calculated as different fluorescence intensity ratios, allowed effective discrimination between water types in connection with the proximity of different contamination sources.

## 2. MATERIALS & METHODS

Water samples have been collected from wells located in Vârtoapele de Jos village, Teleorman County, Romania, in August 2011. The sampling period is important, as the summer time is characterized by a significant impact of anthropogenic activities (irrigation and other household activities) on water quality. We randomly selected 12 water wells characterized by a variety of natural conditions (drainage condition, soil and slope) and different interactions with anthropogenic activities (livestock, crops, domestic activities, and cemetery). The groundwater level has ranged from 70–2950 cm, and the water thickness in wells varied from 205 to 550 cm.

Groundwater contamination depends on specific site characteristics such as: distance from the contamination source, well depth and water layer depth, residence time of the water in the unsaturated area, presence of clay and organic matter in the unsaturated area, decomposition potential of a particular contaminant, amount of precipitation that affects recharge, evaporation, and uncontrolled waste disposal.

Table 1 gives details of the proximity of different contamination sources and well characteristics: groundwater level ( $L$ ) and water layer depth ( $D$ ).

Table 1

General characteristics of well surrounding areas

Sample	Main feature of neighborhood areas	$L/D$ [cm]
H 1	Sheepfold (200-300 sheeps and goats) - poor management of organic wastes; stagnant water due to existing clay soil.	150 / 308
H 2	Small vegetable garden, where the owners use manure as soil fertilizer, aluvial soil (sand texture)	142 / 490
H 3	Sheepfold (200-300 sheeps and goats) at 450 meters; hydraulic condition favors the water stagnation, clay soil.	115 / 205

Table 1 (continued)

H 4	Pollutants from few dwellings; stagnant waters derived from the upstream area and the slopes	70 / 445
H 5	Small sheepfold (100 sheeps and goats) and some dwellings without sewage system.	217 / 213
H 6	Middle of the rural district with dwellings without connection of sewage network- sediment accumulation area.	126 / 510
H 7	Peripheral rural area at higher altitude than most of the village area; infiltration from crops and sheepfold activities.	980 / 300
H 8	Peripheral rural areas, characterized by the infiltration from intensive agriculture, sheepfolds and dwellings.	2950 / 550
H 9	Low density residential areas, situated close to a river that drain the settlements.	262 / 236
H 10	Low density dwellings area without connection to sewage network, with drainage influenced by the presence of road.	232 / 435
H 11	Peripheral rural area, situated in high altitude area with infiltration from crops, dwelling and cemetery.	1240 / 475
H 12	Peripheral area of the settlement, closer to a swampy area, used for domestic waste disposal.	218 / 305

Fluorescence spectra were recorded, in the form of excitation–emission matrices (EEMs), with spectrofluorimeter FLS-920 Edinburgh Instruments, using a Xe lamp of 450 W. The fluorescence signal was collected as a function of both excitation and emission wavelengths, incremented from 230 to 400 nm with 10 nm intervals for excitation and from 250 to 500 nm with 1 nm intervals for emission. This creates a data set with three dimensions: excitation wavelength, emission wavelength and intensity. Darker colors on the map indicate higher fluorescence intensity, proportional to the amount of fluorophores in the sample. The recorded data were treated using ORIGIN 7.5 software.

### 3. RESULTS AND DISSCUSSIONS

Fluorescence EEMs for twelve wells (H1-H12) are illustrated in Fig. 1, with the emission wavelengths plotted on the X axis and excitation wavelengths on the Y axis. For comparison purpose, all maps were normalized at the maximum fluorescence intensity value. Specific fluorescence bands of protein-like fluorophores were recorded in the emission range 310–340 nm, at excitation wavelengths of 240 and 270 nm, while the humic substances appeared in the two emission domains of 380–440 nm with excitation between 240–250 nm (A maximum) and 310–340 nm (C maximum). Peak A is considered to have a primary terrestrial source [5], whereas peak C is increasingly attributed to microbially and chemically reprocessed DOM [6].

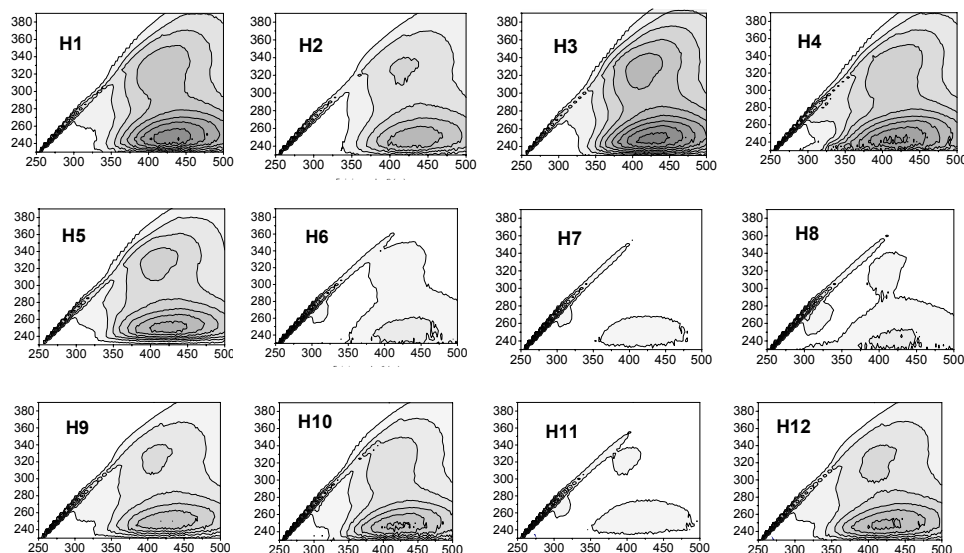


Fig. 1 – EEM for well water samples as described in Table 1,  
With emission wavelengths on X axis and excitation wavelengths on Y axis.

EEM signatures contained many of the characteristic peaks commonly observed in water samples and show that humic substances are the dominant fluorophores. However, some DOM fractions appeared to originate from *in situ* microbiological activity that could be indicative of contamination with domestic discharges and nitrates infiltration from agriculture. As noted in other studies [5], clean water generally exhibits low fluorescence intensity corresponding mostly to the humic-like substances. High humic-like fluorescence was recorded for samples H1-H3 indicating a high concentration of organic matter with a major degree of humification. The wells H1-H3 were located in the proximity of animal and human waste sources, which usually induce a high quantity of bacterial loads in the water. However, fluorescence fingerprints have shown an insignificant microbial fraction at these samples, which could be explained by the presence of clay and sand in the soil surrounding. According to the studies of Szabo *et al.* [11], clay minerals acted as water treatment agents for the removal of organic pollutants. It could be assumed that clay minerals bind with greater affinity to bacteria, preventing the entrance of contaminants into the water system. This fact was supported by the fluorescence fingerprint of the sample collected from well H4, which presented a high quantity of microbial fraction. Well H4 had a groundwater level closer to the surface (70 cm) compared to the other wells and was potentially more exposed to organic contamination sources. Unlike wells H1-H3, the soil surrounding well H4 did not present any clay to function as a natural barrier against water bacterial contamination. However, more studies are needed to identify the exact routes for microbial reduction.

For other wells, H4, H5, H9 and H12 high fluorescence intensity of humic-like components, both A and C, and low contribution from microbial components could be seen. Factors like pollutants from dwellings and domestic waste disposal, together with hydromorphology of soil with stagnant waters contributed to the increase of organic pollutants from the upstream area. Small rivers, swampy areas and rain infiltration were also responsible for the high variation of pollutants concentration.

For samples collected from deep wells, H6-H8 and H11, situated at high altitude area in village, low fluorescence intensities were obtained, but with a significant microbial component relative to the humic fraction. The high altitude is determined by the recent sediment accumulation processes which had not modified the hydrogeological features of the area. Beside the mineral structure, this sediment had an important organic part, which influenced the concentration of nutrients in ground water. Thus, microbial components were present in the samples due to soil characteristics, which facilitated the infiltration of leachable farm wastes and household discharges. The results were conclusive with Baker [3] who has obtained similar fluorescence fingerprint for farm wastes water leachates.

To assess the relevant water properties based on the specific fluorescence emission some fluorescence indices were used: the fluorescence index (FI), denoting DOM origin [6]; humification index HIX [12], used to estimate the degree of DOM maturation; the biological index, BIX [13], dedicated the characterisation of autochthonous inputs and Try/C ratio [3], for the balance of tryptophan and humic substances. Table 2 provides a summary of the range of FI, HIX, BIX, and Try/C indices for wells water samples examined in this study.

Since the FI is an indicator of precursory material, values ranging from 1.39 to 1.61 indicated that all samples maintained a terrestrially-derived signature with low or medium bacterial fraction presence. Birdwell and Engel [14] found FI values above the threshold for significant microbial DOM, showing the presence of natural autochthonous production. Therefore, deeper wells could also contain a minor natural load of microbial DOM, apart from organic contaminants. According to literature [12, 13], for our samples HIX values ranging between 1.2 and 3 referred to water samples with DOM of bacterial origin; between 4.3–4.7 samples contained DOM with weak humic character and important recent microbial component and between 7.2 and 8.3 with an important humic character and weak recent autochthonous component. Samples that presented strong autochthonous fraction corresponded to 0.8–1 values of BIX, while the biological or aquatic bacterial origin was indicated by values of BIX >1. Both HIX and BIX correlated well with the sampling sites characteristics. Using these parameters, we succeeded to characterize the organic matter present in samples, in connection with the characteristics of wells surrounding areas; thus, shallow depth wells showed the highest humic concentration denoted by the highest HIX values, while samples collected from wells nearby stagnant waters and/or household discharges sources were characterized by the lowest HIX and highest BIX values.

Table 2

Fluorescence indices

Sample	FI	HIX	BIX	Try/C	Category
H1	1.39	7.27	0.86	0.19	A
H2	1.39	8.25	0.83	0.15	A
H3	1.25	7.66	0.90	0.14	A
H4	1.43	4.35	0.92	0.32	B
H5	1.45	4.67	0.95	0.28	B
H6	1.49	2.88	0.90	0.71	C
H7	1.60	1.65	1.19	1.31	C
H8	1.50	1.61	0.97	1.16	C
H9	1.41	4.19	0.93	0.37	B
H10	1.54	1.20	1.13	1.36	C
H11	1.61	1.82	1.13	1.06	C
H12	1.41	4.65	0.93	0.32	B

Both spectral fingerprints and fluorescence indices classified the wells in three groups. This division was based on the soil quality, depth of wells and groundwater levels that allowed the accumulation of contaminants in groundwater. The analysis of groundwater DOM fluorescence could provide information on how surface water infiltrates into aquifers and on the groundwater quality. Organic matter character of well water might be more rigorously studied by analyzing the relative proportion of the terrestrial and microbial fractions in the water systems. The two main fluorescence components, protein-like (Try) and humic-like (C) were found in different proportion in the samples, as can be seen in Fig. 2. Samples were divided in three categories: first one (named A) with protein-like percentage of up to 18% (samples H1, H2 and H3), the second one (named B) with medium content of microbial component in proportion between 20–22% (H4, H5, H9, H12) and samples (category C) with predominant microbial content 40–60 % (H6, H7, H8, H10, H11).

Category A samples presented HIX values between 7.3–8.3 and BIX in the range of 0.83–0.9, meaning samples with important humic character and recent autohtonous component. Samples belonging to small water depth wells, showed a high degree of humification and aromaticity due to light exposure or clay soil (H1, H2, H3). The close proximity of sheepfold and households manure was the origin of the recent minor bacterial component in samples, due to the clay content of soils, which prevented organic matter and contaminant accumulation.

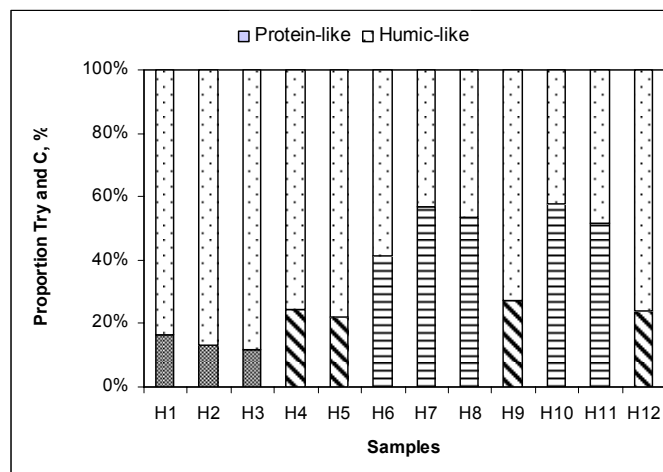
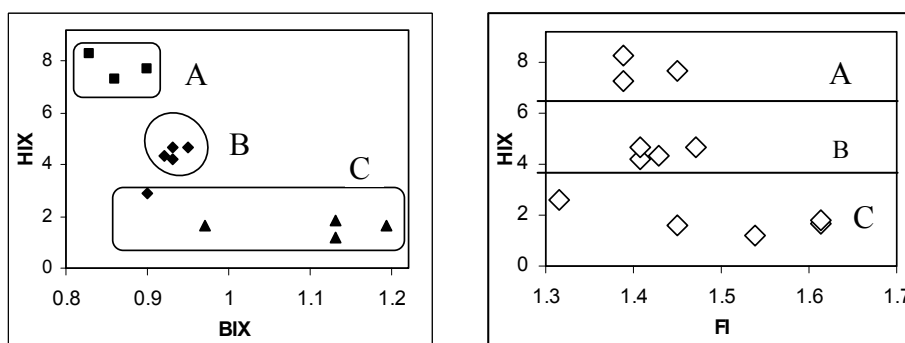


Fig. 2 – Proportion of protein-like and humic-like components in samples.

The dependence of HIX on fluorescence parameters BIX and FI is illustrated in Fig. 3 indicating the specific domains for the three categories of samples.



Figs. 3 – HIX dependence on BIX and FI, A (H1 –H3); B (H4, H5, H9, H12) and C (H6, H7, H8, H10, H11).

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Category B consisted of wells H4, H5, H9 and H12. All samples had HIX <5 denoting fresh DOM derived from decomposition of terrestrial or animal manure,

as also identified in other studies [15, 16]. The wells presented medium depth and were situated in residential area where contamination came from microbial accumulation in stagnant waters, rivers, household and animal farm discharges.

The low HIX (1.2–2.9) and high BIX ( $> 0.97$ ) values for samples H6, H7, H8, H10, H11 (category C) indicated fluorescence of microbiological activity and less humified aquatic humic substances, possibly due to a lack of exposure to solar radiation, as similarly detected by [14]. Higher protein-like than humic-like fluorescence was detected, as can be seen in Fig. 2, in agreement with results obtained for karst spring and cave water by other authors [14, 16]. An important characteristic for all these wells was their medium and high water depth and the proximity of different contamination sources, such as discharges from residential areas and adjacent agriculture, sheepfold and surrounding dwellings. The explanation was also based on the relief, hydrological and hydrogeological features of the study area.

The intrinsic fluorescence technique could be used complementary to other groundwater investigation methods in providing an overview on the surrounding area that influences the water quality.

#### 4. CONCLUSIONS

In this study, three-dimensional fluorescence spectroscopy was used to investigate the dissolved organic matter specific for some wells situated in a rural area. Some of the analysed water samples revealed an important humic character of DOM with high degree of aromaticity, at the same time others displayed weak humic character and important recent microbial component attributed to leachate infiltration from on-site sewage disposal system, drainage configuration and mixture between clay and sand texture of the soil.

Three distinct groups of wells were identified using fluorescence indices FI, HIX and BIX. The distinctions derived from combinations of various factors such as: well characteristics, sources of contamination from nearby areas and soil characteristics. Although more environmental degradation sources (*e.g.* domestic, farmland, crops and cemetery) influenced low depth well water, the major water quality problems appeared in the medium and high depth strata. The explanation was based on the relief, hydrological and hydrogeological features of the studied area. Thus, high altitude was determined by the recent sediment accumulation processes which had not modified the hydrogeological feature of the area. Beside the mineral structure, this sediment had an important organic part which influenced the concentration of nutrients in ground water.

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## REFERENCES

1. D.J. Lapworth, N. Baran, M.E. Stuart, R.S. Ward, *Emerging organic contaminants in groundwater: A review of sources, fate and occurrence*, Environmental Pollution, **163**, 287–303 (2012).
2. A. Rotaru, P. Răileanu, *Groundwater contamination from waste storage works*, Environ. Eng. Manag., J, **7**, 6, 731–735 (2008).
3. A. Baker, *Fluorescence properties of some farm wastes: Implications for water quality monitoring*, Water Res., **36**, 189–194 (2002).
4. C.A. Tudor, I.C. Ioja, A. Hersperger, I. Pătru-Stupariu, *Is the residential land use incompatible with cemeteries location? Assessing the attitudes of urban residents*, Carpath. J. Earth Env., **8**, 2, 153–162 (2013).
5. N. Hudson, A. Bake, D. Reynolds, *Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters – a review*, River Research and Applications, **23**, 631–649 (2007).
6. D.M. McKnight, E.W. Boyer, P.K. Westerhoff, P.T. Doran, T. Kulbe, D.T., Andersen, *Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity*, Limnol. Oceanogr., **46**, 38–48 (2001).
7. E.M. Carstea, A. Baker, M. Bieroza, D. Reynolds, *Continuous fluorescence excitation emission matrix of river organic matter*, Water Research, **44**, 18, 5356–5366 (2010).
8. D.J. Lapworth, D.C. Gooddy, A.S. Butcher, B.L. Morris, *Tracing groundwater flow and sources of organic carbon in sandstone aquifers using fluorescence properties of dissolved organic matter (DOM)*, Applied Geochemistry, **23**, 3384–3390 (2008).
9. L. Ghervase, C. Ioja, E.M. Carstea, D. Savastru, *Human daily activities reflected by the ecological state of natural water resources*, Environ. Eng. Manag. J, **11**, 3, 567–571 (2012).
10. J. Bridgeman, A. Baker, C. Carliell-Marquet, E. Carstea, *Determination of changes in wastewater quality through a treatment works using fluorescence spectroscopy*, Environmental Technology, In press 2013.
11. C. E. Szabó, K. Vajda, G. Veréb, A. Dombi, K. Mogyorósi, I. Ábrahám, M. Májer, *Removal of organic pollutants in model water and thermal wastewater using clay minerals*, J Environ Sci Health A, Tox Hazard Subst Environ Eng, **46**, 1346–1356 (2011).
12. T. Ohno, *Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter*, Environmental Science and Technology, **36**, 742–746 (2002).
13. L. Huguet, S. Vacher, S. Relexans, J.M. Saubusse, E. Froidefond, E. Parlanti, *Properties of fluorescent dissolved organic matter in the Gironde Estuary*, Organic Geochemistry, **40**, 706–719 (2009).
14. J. E. Birdwell, A. S. Engel, *Characterization of dissolved organic matter in cave and spring waters using UV-Vis absorbance and fluorescence spectroscopy*, Organic Geochemistry, **41**, 270–280, (2010).
15. T. Ohno, and R. Bro, *Dissolved organic matter characterization using multiway spectral decomposition of fluorescence landscapes*, Soil Science Society of America Journal, **70**, 2028–2037 (2006).
16. M. Mudarra, B. Andreo, A. Baker, *Characterisation of dissolved organic matter in karst spring waters using intrinsic fluorescence: Relationship with infiltration processes*, Sci. Total Environ., **409**, 18, 3448–3462 (2011).