

Microbial Changes in the Fluorescence Character of Natural Organic Matter from a Wastewater Source

Rémi Riopel^{1,2}, Stefan Siemann¹, François Caron^{1,3*}

¹Chemistry and Biochemistry Department, Laurentian University, Sudbury, Canada

²Radiological Protection Research and Instrumentation Branch, Canadian Nuclear Laboratories (CNL), Chalk River, Canada

³School of the Environment, Laurentian University, Sudbury, Canada

Email: fcaron@laurentian.ca

Received 5 February 2016; accepted 23 August 2016; published 26 August 2016

Copyright © 2016 by authors and Scientific Research Publishing Inc.

This work is licensed under the Creative Commons Attribution International License (CC BY).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

Natural Organic Matter (NOM) is a mixture of aromatic and aliphatic organic compounds of natural origin in any type of aquatic system. Human activities impact the constituents of NOM, from its production to its fate, particularly in the treatment of domestic waste waters. In this work, the impact of microorganisms isolated from a Waste Water Treatment Plant (WWTP) was investigated to determine the fate of NOM fractions in raw sewage, using fluorescence spectroscopy. Wastewater samples were taken at three different times from a WWTP, and incubated for 4 days under two treatments: 1) “raw sewage”, and 2) “spiked”, *i.e.*, the same raw sewage, spiked with bacteria previously isolated from the WWTP. The incubated waters were analyzed by fluorescence spectroscopy, digitally resolved into NOM components: humic- and fulvic-like, and two types of protein-like, *i.e.*, tryptophan- and tyrosine-like, using a Parallel Factor Analysis routine (PARAFAC). The results demonstrate that the “spiked” samples showed the largest changes with incubation time. The signals of the tryptophan- and tyrosine-like components decreased, suggesting a net microbial digestion of proteinaceous material. In contrast, the fulvic-like signals, and to some extent, the humic-like signals increased, suggesting the production of the associated molecular materials during the incubation period. This study provides direct evidence of human impact on the make-up of NOM: the cultures of microbes found at a WWTP consume the proteinaceous material, whereas humic-like and fulvic-like materials are produced.

Keywords

Natural Organic Matter, Wastewater, Microbial Utilization, Fulvic-Like, Protein-Like, Incubation,

*Corresponding author.

How to cite this paper: Riopel, R., Siemann, S. and Caron, F. (2016) Microbial Changes in the Fluorescence Character of Natural Organic Matter from a Wastewater Source. *Journal of Water Resource and Protection*, 8, 873-883.
<http://dx.doi.org/10.4236/jwarp.2016.89072>

Fluorescence, PARAFAC

1. Introduction

Natural Organic Matter (NOM) is a term used to describe the material derived from the naturally occurring degradation of organic compounds, and it is present in all natural waters. NOM is a collection of aromatic and aliphatic macromolecules ranging from a few hundred to several thousand of Daltons (Da) in size. Sources of NOM constituents include accumulated organic matter, the decay of higher plants, grasses, leaf litter and other living material, all of which are found in all types of waters, such as lakes, peat bogs, rivers, and marine environments [1]-[11]. In addition to the decomposition of already formed organic matter, normal biological functions also produce NOM through excretion, leaching and other metabolic functions [12]-[15]. Human activities can produce other forms of NOM, and potentially modify existing NOM in aquatic systems [15]-[17], either indirectly through land use (e.g., deforestation, agriculture, urbanization, etc.) or more directly through processes such as landfilling, composting [18] [19], or waste water treatment [20] [21].

In recent years, fluorescence spectroscopy has been applied to track changes in NOM constituents through aquatic and environmental processes [16] [21]-[25]. The technique is fast, non-destructive and non-invasive, and can yield specific information on the classes of NOM. Fluorescence signals are usually resolved using digital techniques; one of the most utilized is Parallel Factor Analysis (PARAFAC; see [1]). Resolved signals have been reported as Humic-like, Fulvic-like, and Protein-like [16] [21] [24]-[28].

The current study complements our previous work [21], on the scale of a Waste Water Treatment Plant (WWTP) at a mid-sized city in Northern Ontario, Canada, in which fluorescence/PARAFAC was applied to track the changes of the NOM between raw sewage and effluent samples of the plant. The work focused on the dynamics of humic-like, fulvic-like and protein-like components. The work has shown a preferential degradation of the protein-like components, and a moderate degradation of humic-like material. On the other hand, fulvic-like material did not degrade appreciably; rather, it showed a slight apparent increase at the effluent. That work lacked specific information on the proteinaceous material degraded (tyrosine- vs. tryptophan-like), and other information on the production or utilization of fulvic- and humic-like material.

Because of the lack of specific information on the degradation or production of specific components, we have decided to complement the earlier work by incubating incoming raw waste water in a laboratory environment. Therefore, the main objective of this work is to determine if microbial consortia found in raw sewage from a WWTP produces or decreases specific NOM fractions from raw sewage. Three different samples, taken within an 8-week span, were incubated immediately after collection, over a 4-day period under two different treatments conditions: 1) incoming raw sewage; and 2) the same sewage water, inoculated with bacteria previously isolated from an earlier sampling at the WWTP. These solutions were monitored for changes in NOM components using fluorescence spectroscopy in combination with PARAFAC spectral resolution. The benefit of this work is to show how human intervention, such as a wastewater treatment plant, influences the quality of NOM, which leads to a better understanding of pollution control.

2. Experimental Procedures

2.1. Bacteria Sampling & Isolation

A composite raw sewage sample was taken on May 5th 2011 from the raw sewage of the WWTP in Sudbury, Ontario, for seeding purposes (see [21] for a description of the plant). Two raw sewage aliquots of 10 mL each were transferred to separate flasks containing 500 mL of pre-autoclaved Luria-Bertani broth (BioShop, Burlington, ON). These were then incubated for 20 hours at 37°C with agitation at 180 rpm in an Innova 4300 incubator shaker (New Brunswick, Edison, NJ). Both cultures were then combined into a 1 L bottle and centrifuged at 2250 g for 1 hour at 4°C using an Avanti J-20 XPI cooled centrifuge (Beckman Coulter, Mississauga, ON). After supernatant removal, the cell suspension (~25 mL) was supplemented with 5 mL of sterile glycerol, then split into 6 aliquots of ~5 mL each and stored at -80°C. Cell viability was assessed by streaking out 50 µL of the suspension onto LB agar plates, followed by incubation overnight at 37°C.

2.2. Sampling, Incubations and Processing

Three separate samples of incoming raw water were taken on May 9th, June 20th and July 4th 2011. For each one of these dates, a fresh 1 L sample was taken and split into pre-cleaned 500 mL polycarbonate bottles (2×500 mL subsamples; PETE by I-Chem), transported to our lab in a dark cool container, and then stored in a refrigerator until use (typically within the day). Two subsamples were used for each one of the three incubations: 1) “raw sewage”, which consisted of filtered incoming raw wastewater ($0.45 \mu\text{m}$ pore size, A/G technologies); and 2) the “spiked” sample, which was the second aliquot of raw sewage water, filtered ($0.45 \mu\text{m}$), and supplemented with a 5 mL aliquot of the bacterial cell suspension isolated earlier (section 2.1).

The two subsamples (1) and (2) were set into separate autoclaved 1-L lecture bottles, capped and wrapped in tin foil to keep in subdued darkness. Subsample (1) was left as-is in the lecture bottle, whereas the lecture bottle of subsample (2) was attached to a recirculation system set-up (**Figure 1**). The cartridge (A/G technologies, $0.45 \mu\text{m}$ pore size) and the recirculation system were pre-treated with detergent (Sparkleen, Fisher Scientific), thoroughly rinsed with Millipore water, and pre-sanitized with a 1/100 solution of commercial bleach (5% NaOCl). This cleaning was repeated prior to introducing the next cell suspension. Rinse water (retentate and filtrate) were monitored with a Dohrmann DC-80 carbon analyzer to ensure low TOC levels before incubation.

Aliquots (~ 40 mL) were withdrawn directly from the bottom outlet of the lecture bottle (“raw sewage” sample), or from the filter outlet for the “spiked” sample (“Filtrate” in **Figure 1**). These aliquots were taken once daily over the 4-day incubation period (96 hours). The aliquots of the “raw sewage” incubated subsamples were then filtered with a standard $0.45 \mu\text{m}$ Durapore filter (Millipore), whereas the subsamples from the “spiked” set up did not require additional filtration. The filtered aliquots were transferred into pre-cleaned, Teflon-backed stoppered 40 mL amber glass bottles (I-Chem) for immediate fluorescence analysis.

2.3. Fluorescence Analysis

An Olis RSM 1000 F1 spectrofluorometer controlled by the OlisGlobalWorks software (version 4.8.17, Bogart, GA) was used for fluorescence analysis. Standard 1-cm quartz cuvettes (Starna Cells, Atascadero, CA) were used. The raw spectra were downloaded as a numerical file, and saved using ExcelTM spreadsheets. The data was processed with an in-house interface Kile routine (Smith, unpubl.) to remove the Rayleigh/ Tyndall scattering lines, and then resolved with PARAFAC to isolate individual components of the fluorescence spectrum. Additional details of the analysis and the spectral resolution are given in [21].

The PARAFAC routine yielded these spectral regions: humic-like, fulvic-like and protein-like, based on earlier work [21] [25] [29] [30] and established literature [3] [16] [24] [26]. We have further refined the protein-like regions in PARAFAC into tryptophan- and tyrosine-like signals which correspond to the protein-like B and T

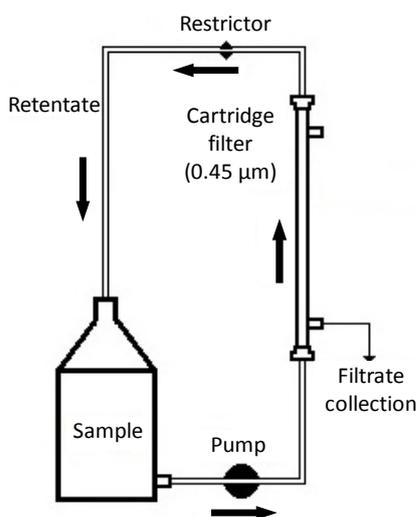


Figure 1. Experimental setup of the recirculation system for the “Spiked” samples, showing the filtrate sampling point.

peaks reported by [3], respectively. This distinction was important in this work, as the fluorescence signal of tryptophan-like material is sensitive to its microenvironment: its emission maximum is at ~330 nm if tryptophan-like moieties are surrounded by hydrophobic structures (*i.e.*, in a non-polar microenvironment such as in folded or undegraded proteins), whereas the emission maximum is near 350 nm if the constituents are in a polar, hydrophilic microenvironment such as that encountered in degraded proteins [31] [32]. Tyrosine-like material, on the other hand, does not exhibit this behaviour [33]. In addition, the excitation wavelength of the latter is ~5 - 10 nm lower than that of tryptophan, and the emission regions are different, making it possible to distinguish tyrosine- and tryptophan-like materials from one another in PARAFAC.

2.4. Standards & Corrections

Salicylic acid (SA) was our primary standard to monitor instrument drift, and to normalize the signal intensities [21] [34]. We also prepared solutions of L-tryptophan (Eastman Organic Chemicals) and L-tyrosine (BDH Chemicals) to validate the signals of the protein-like region for processing the files in PARAFAC (Ex/Em of ~280/350 and ~280/310, respectively). All these solutions were prepared weekly, in Millipore water, at a concentration of 10^{-5} M. The blanks were fresh de-ionized water (Millipore). The absolute fluorescence units may or may not be comparable to other studies; however, our results are normalized to these standards for an internally consistent set of data.

3. Results

The fluorescence signals of the components (isolated by PARAFAC) of the three waste water samples (May, June and July), and the two treatments, produced similar features throughout the incubations (Figure 2). The component centroids were found at distinct emission and excitation wavelengths (Ex/Em). For instance, the humic-like (~340/420 nm) and fulvic-like (~320/380 nm) signals were generally broad and almost featureless, with overlapping portions. The two protein-like signals were assigned to tryptophan-like (~280/340 nm) and tyrosine-like (~275/310 nm) material. Although these were close together, they were fairly narrow and intense, and hence sufficiently distinct from one another to classify them accordingly.

Despite these similarities, the three samples exhibited different relative signal intensities (Figure 3), suggesting different NOM feedstocks (Figure 3). This could be due partially to differences in diet of inhabitants, different NOM in urban runoff, or different seasonal sources of NOM. Although no seasonal trend was found [21], the May sample was taken after the snowmelt but before the growing season, whereas the June and July samples were well into the growing season. We also noted that the tyrosine-like signals were low but could be easily distinguished from those of tryptophan-like signals for these three samples. Figure 4 shows an example of the changes in relative fluorescence intensities for the three samples as a function of time for the two incubation modes (“raw sewage” and “spiked”). For all three samples, differences in the NOM composition were observed between the two incubation modes, and also with time.

3.1. Incubation of Raw Sewage

The relative distribution of components in the “raw sewage” samples was different between day 0 and day 1 for all three samples, possibly due to a change in initial conditions (temperature change, filtering, etc.). Only small changes were apparent with incubation time between day 1 and 4 for all three samples, except perhaps for decreases in tryptophan-like signals. The microbial populations in the raw water might have been too low, not very active in substrate utilization, or not producing NOM to a significant extent.

3.2. Incubation of “Spiked” Sewage

The relative intensities of humic- and fulvic-like material remained similar or slightly smaller in all three “spiked” samples at day 0, compared to their corresponding “raw sewage” samples. On the other hand, the signal intensities of both protein materials (tryptophan- and tyrosine-like) increased several-fold. This increase could be caused by the initial introduction of protein material during the cell inoculation. Later on with incubation times, we have observed a systematic decrease of both tryptophan- and tyrosine-like signals (although the decrease was not so clear for the incubation of the May sample). This decrease could be attributed to the bacterial consumption of protein material, until its concentration was too low to be a target nu-

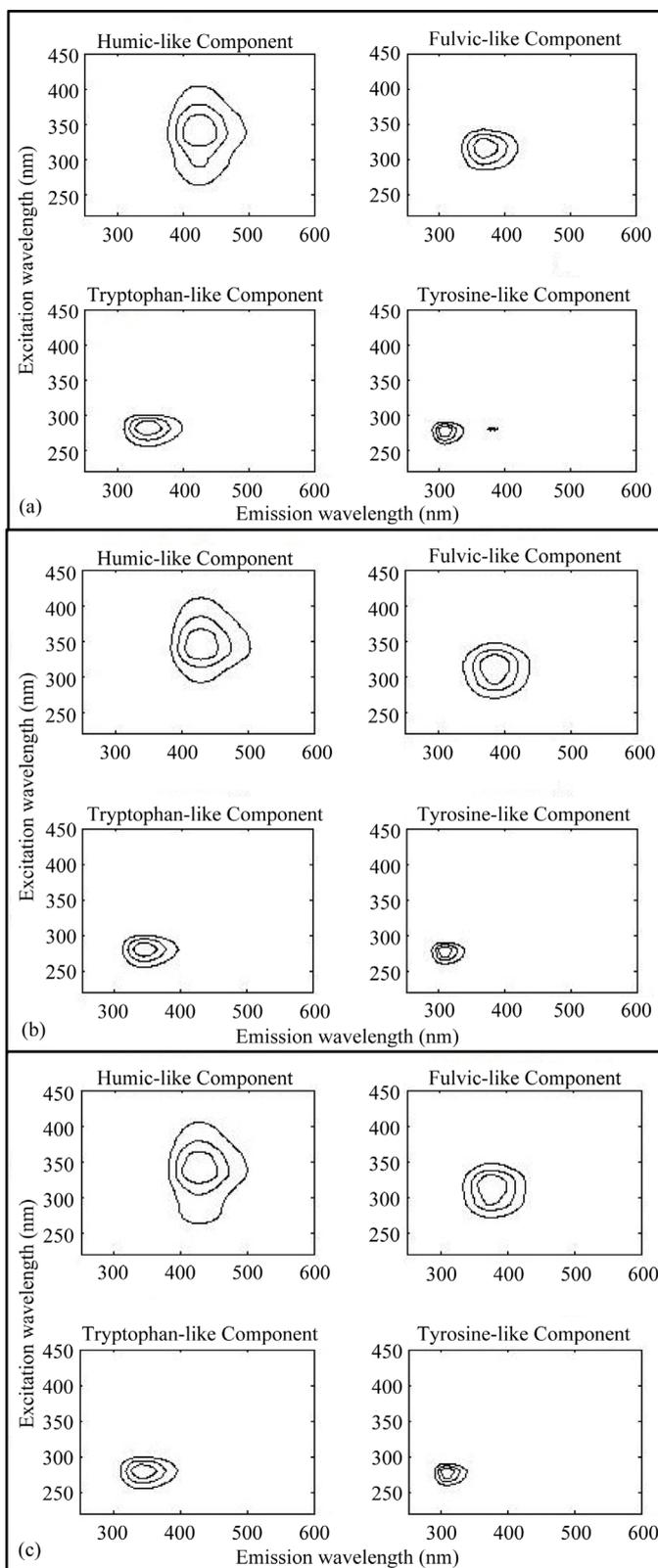


Figure 2. Plots of excitation/emission components (Ex/Em, in nm) after resolution with PARAFAC: samples from May (a); June (b); and July (c). Each plot was obtained for a sample, one analysis per day, for the 5 incubation times, and two incubation modes (“raw sewage”, “spiked”). The concentric lines indicate signal intensities (in relative fluorescence units).

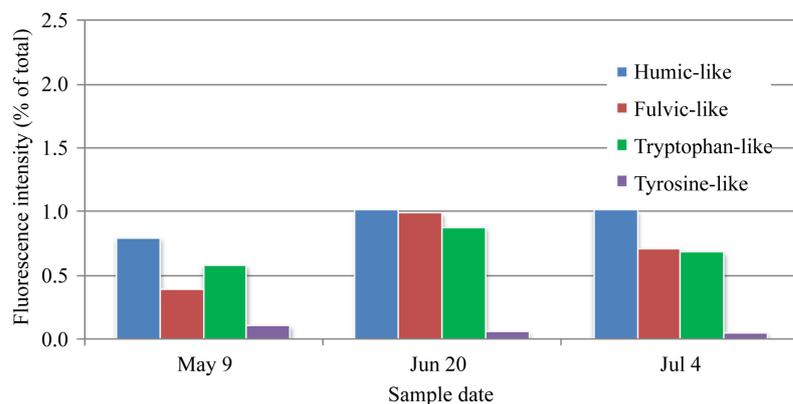


Figure 3. Fluorescence signal intensities of the three samples, after PARAFAC resolution (the signal intensities are corrected with salicylic acid for internal consistency—see text).

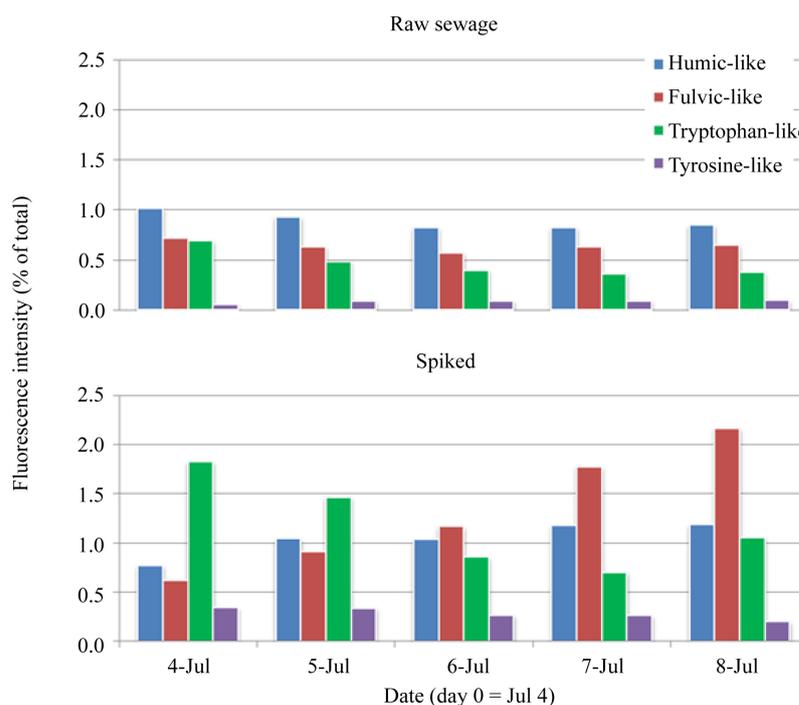


Figure 4. Example of the changes in relative fluorescence intensity of the NOM components as a function of the incubation period (July sample).

trient. The consumption of both types of protein-like material has been reported in our earlier study, at the scale of the WWTP [21]. Increases of fulvic-like and, to a lesser extent, humic-like material were observed for all three samples.

4. Discussion

The type of NOM consumed or produced by microbial activity is not well known for any type of water (lakes, sediments, waste waters, etc.). The bacterial consortia present in wastewaters are likely mixed, which means that they have different abilities to adapt as a response to conditions [4] [35]–[37]. Despite the differences in the original NOM feedstock, the relative fluorescence intensities of the components have become almost identical for the three samples at the end of the incubation period (Figure 5). This has implications for treatment plants using activated sludge: we have reported that our three samples, taken in a time span of ~8 weeks, had different

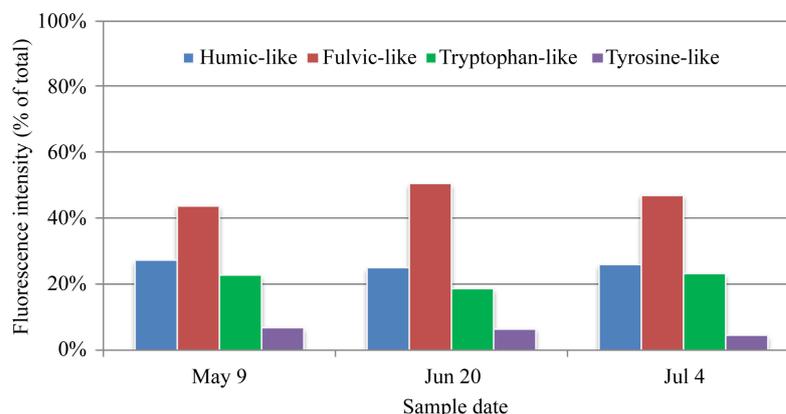


Figure 5. Comparison of the relative fluorescence signal intensities for the 3 “spiked” samples, at the end of the 4-day incubation period.

original NOM compositions (Figure 3). This suggests that the microbes produce NOM with similar fluorescence signals upon digestion, and likely similar compositions. We believe this applies to the scale of a treatment plant, as also suggested earlier [21]. Our study does not differentiate between NOM feedstock utilization, or NOM from decomposition [15]; our incubations are likely a combination of both.

The changes in fluorescence intensity in the “raw sewage” samples were minimal for the 4-day incubation period of this experiment (Table 1). There is undoubtedly microbial and/or other activity in these samples; it is possible that the incubation period is too short to reveal any substantial changes in the fluorescent NOM. On the other hand, in our “spiked” experiment, the samples provided some key and unique results. These samples showed a substantial increase in fulvic-like intensity with incubation time for the three samples, and a smaller but noticeable increase in humic-like signal (Figure 6). Decreases in tryptophan- and tyrosine-like intensities were also observed (Table 1), albeit the signal intensity was smaller for the latter.

Two mechanisms could be suggested: 1) substrate utilization, and 2) decomposition/respiration. For mechanism (1), the spiked material may dominate early on in the incubations. Decreases in tryptophan- and tyrosine-like signals can be interpreted as the net microbial assimilation or degradation of protein-like material. The relative changes of the proteinaceous signals (tyrosine- and tryptophan-like) were different, suggesting that the bacterial consortium in the cultures degraded the components differently. For mechanism (2), NOM produced by decomposition or respiration would be produced later in the incubations, at the expense of protein-like material (mostly tryptophan-like). The substances produced have humic- and fulvic-like properties. Our suggestions for these two mechanisms are reasonable in this context, and are consistent with the experiments of Kang *et al.* [15], and in constructed wetland systems [38]. This is also consistent with our earlier work [21], in which substantial decreases in labile protein-like material were reported at a WWTP outlet.

These observations are unique and original, providing clear and direct evidence that material of humic- and fulvic-like properties are produced by bacteria in raw sewage, and that proteinaceous material (tryptophan- and tyrosine-like) is consumed. The consumption of proteinaceous material at the scale of waste water plants has been reported before [21] [38]-[40]. The direct production of fulvic-like material has been suggested by Kang *et al.* (2016) [15] in their bioreactor, for the senescent or respiration stage. Similarly, on a larger scale, Riopel *et al.* (2014) [21] suggested the formation of fulvic- and humic-like material in a WWTP; Yao *et al.*, (2016) have reported a decrease of tryptophan-like and an increase of humification in constructed wetlands [38].

The formation of humic substances (humic and fulvic acids) is not a straightforward process, and it is different between soils, sediments, lakes, etc. These substances can originate from the agglomeration and decay of lignin precursors (retaining some degree of aromaticity), or from the polycondensation of glycerides, among others [41]. Stevenson (1982) [42] has also postulated that microorganisms can synthesize polyphenols from non-lignin carbon sources which could be converted into quinones, and combine with other metabolites, such as amino acids or peptides, to form humic polymers. The fulvic acids could be created first, since they are smaller in structures. It is conceivable that fulvic acids agglomerate into the larger humic acids, aided by microbial decay and re-condensation. In our cultures, the incubation time might be too short to observe this directly.

The production of NOM with humic-like and fulvic-like characteristics, along with the consumption of ma-

Table 1. Relative changes in fluorescence signal between day 1 and day 4 (“+” is a relative signal increase, “-” is a decrease). Note that the comparison is done with the second incubation date (day 1) to exclude the potential bias due to the lag phase at day 0, usually present upon inoculation.

	Humic-like	Fulvic-like	Tryptophan-like	Tyrosine-like
Raw sewage sample				
May	13%	13%	6%	10%
June	-30%	-13%	-18%	-2%
July	-9%	3%	-22%	6%
Spiked sample				
May	5%	372%	-1%	-35%
June	13%	63%	-46%	-20%
July	14%	137%	-28%	-41%

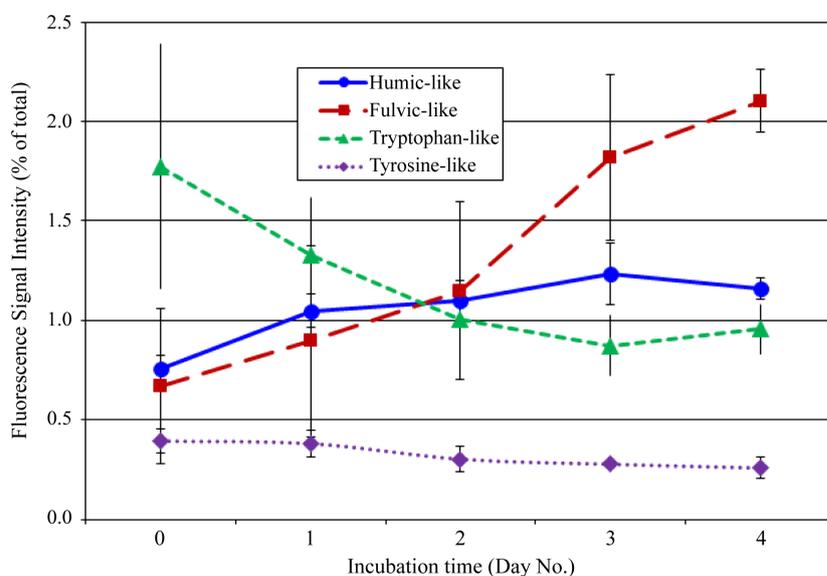


Figure 6. Averages of the fluorescence signal intensities for the four components from the “spiked” samples, for all three samples. The error bars are calculated “standard deviations” and represent variabilities around the mathematical averages, with the understanding that the sample feedstock was different among samples (see text).

terial with protein-like characteristics, has also been reported before for extracellular organic matter in algae cultures [43]. Riopel *et al.* (2014) [21] have also shown a direct decrease of protein-like material between the inlet and outlet of a WWTP, and a potential increase of fulvic-like material. However, they have reported a decrease of humic-like material, which is in contrast to the results of the current study. Other evidence of the production of protein-like material, at the expense of humic- and fulvic-like material, has been proposed in carbon-poor systems [25] [29] [44], and in human-impacted systems [16]. Other studies [1] [4] [15] have suggested that, even though protein-like material can be a substrate for microbial degradation, it can be produced as well.

Our observations constitute a unique and original contribution to the field of waste treatment, because we are able to demonstrate directly that bacterial consortia from a WWTP change the distribution of NOM. Despite different NOM feedstock, the relative distribution of NOM was similar at the end of the incubations. These conclusions were reached directly from cultures of actual waste water, and complementing earlier work at the scale of a WWTP, hence showing the possibility of wide-ranging implications for treatment plants. This also provides how human activities impact the assimilation, transformation, and production of different types of NOM in nature.

5. Conclusions

Incubations of three different waste water samples were performed over a period of 4 days, under two different pre-treatments: as-is (or “raw sewage”), and after inoculation (“spiked”) with a suspension containing bacterial cells previously isolated from raw sewage. The NOM feedstock was different from these samples, yet the samples spiked with previously isolated bacteria yielded NOM with similar fluorescence signals.

The “spiked” incubations showed an overall increase in the fluorescence signal of the fulvic-like and humic-like materials, and an overall decrease of the tryptophan- and the tyrosine-like materials. These observations suggest that the microbial cultures are consuming the protein-like materials (tryptophan- and tyrosine-like), and producing humic- and fulvic-like materials. Although the specific mechanisms of degradation and/or assimilation are currently unknown, the relative composition of the NOM components is fairly similar, based on the fluorescence signals, suggesting that similar classes of molecules are produced after degradation. Our experiments confirm earlier suggestions that fulvic-like, and to some extent, humic-like material, can be produced in wastewater treatment plants, hence providing an example of NOM production or modification from human activities.

Acknowledgements

We wish to thank D. Miron and C. Thibeault of the Sudbury Waste Water Treatment Plant for site access, use of facilities, and sampling support. This work was partially funded by Laurentian University, with administrative support from C. Mosher (CIMMR, Laurentian University, Sudbury, ON). D.S. Smith (Wilfrid Laurier University) provided the in-house PARAFAC routine to F. Caron.

References

- [1] Cammack, W.K.L., Kalff, J., Prairie, Y.T. and Smith, E.M. (2004) Fluorescent Dissolved Organic Matter in Lakes: Relationships with Heterotrophic Metabolism. *Limnology and Oceanography*, **49**, 2034–2045. <http://dx.doi.org/10.4319/lo.2004.49.6.2034>
- [2] Clark, C.D., Jimenez-Morais, J., Jones II, G., *et al.* (2002) A Time-Resolved Fluorescence Study of Dissolved Organic Matter in a Riverine to Marine Transition Zone. *Marine Chemistry*, **78**, 121–135. [http://dx.doi.org/10.1016/S0304-4203\(02\)00014-2](http://dx.doi.org/10.1016/S0304-4203(02)00014-2)
- [3] Coble, P.G. (1996) Characterization of Marine and Terrestrial DOM in Seawater Using Excitation-Emission Matrix Spectroscopy. *Marine Chemistry*, **51**, 325–346. [http://dx.doi.org/10.1016/0304-4203\(95\)00062-3](http://dx.doi.org/10.1016/0304-4203(95)00062-3)
- [4] Elliott, S., Lead, J.R. and Baker, A. (2006) Characterisation of the Fluorescence from Freshwater, Planktonic Bacteria. *Water Research*, **40**, 2075–2083. <http://dx.doi.org/10.1016/j.watres.2006.03.017>
- [5] Hautala, K., Peuravuori, J. and Pihlaja, K. (2000) Measurement of Aquatic Humus Content by Spectroscopic Analyses. *Water Research*, **34**, 246–258. [http://dx.doi.org/10.1016/S0043-1354\(99\)00137-2](http://dx.doi.org/10.1016/S0043-1354(99)00137-2)
- [6] Her, N., Amy, G., McKnight, D., *et al.* (2003) Characterization of DOM as a Function of MW by Fluorescence EEM and HPLC-SEC Using UVA, DOC, and Fluorescence Detection. *Water Research*, **37**, 4295–4303. [http://dx.doi.org/10.1016/S0043-1354\(03\)00317-8](http://dx.doi.org/10.1016/S0043-1354(03)00317-8)
- [7] Jaffé, R., Boyer, J.N., Lu, X., *et al.* (2004) Source Characterization of Dissolved Organic Matter in a Subtropical Mangrove-Dominated Estuary by Fluorescence Analysis. *Marine Chemistry*, **84**, 195–210. <http://dx.doi.org/10.1016/j.marchem.2003.08.001>
- [8] Katsuyama, M. and Ohte, N. (2002) Determining the Sources of Stormflow from the Fluorescence Properties of Dissolved Organic Carbon in a Forested Headwater Catchment. *Journal of Hydrology*, **268**, 192–202. [http://dx.doi.org/10.1016/S0022-1694\(02\)00175-0](http://dx.doi.org/10.1016/S0022-1694(02)00175-0)
- [9] Mounier, S., Patel, N., Quilici, L., *et al.* (1999) Fluorescence 3D de la matière organique dissoute du fleuve amazon: (Three-Dimensional Fluorescence of the Dissolved Organic Carbon in the Amazon River). *Water Research*, **33**, 1523–1533. [http://dx.doi.org/10.1016/S0043-1354\(98\)00347-9](http://dx.doi.org/10.1016/S0043-1354(98)00347-9)
- [10] Parlanti, E., Wörz, K., Geoffroy, L. and Lamotte, M. (2000) Dissolved Organic Matter Fluorescence Spectroscopy as a Tool to Estimate Biological Activity in a Coastal Zone Submitted to Anthropogenic Inputs. *Organic Geochemistry*, **31**, 1765–1781. [http://dx.doi.org/10.1016/S0146-6380\(00\)00124-8](http://dx.doi.org/10.1016/S0146-6380(00)00124-8)
- [11] Yamashita, Y. and Tanoue, E. (2003) Chemical Characterization of Protein-Like Fluorophores in DOM in Relation to Aromatic Amino Acids. *Marine Chemistry*, **82**, 255–271. [http://dx.doi.org/10.1016/S0304-4203\(03\)00073-2](http://dx.doi.org/10.1016/S0304-4203(03)00073-2)
- [12] Chen, W., Westerhoff, P., Leenheer, J.A. and Booksh, K. (2003) Fluorescence Excitation-Emission Matrix Regional Integration to Quantify Spectra for Dissolved Organic Matter. *Environmental Science and Technology*, **37**, 5701–5710. <http://dx.doi.org/10.1021/es034354c>

- [13] Sheng, G.-P. and Yu, H.-Q. (2006) Characterization of Extracellular Polymeric Substances of Aerobic and Anaerobic Sludge Using Three-Dimensional Excitation and Emission Matrix Fluorescence Spectroscopy. *Water Research*, **40**, 1233-1239. <http://dx.doi.org/10.1016/j.watres.2006.01.023>
- [14] McIntyre, A.M. and Guéguen, C. (2013) Binding Interactions of Algal-Derived Dissolved Organic Matter with Metal Ions. *Chemosphere*, **90**, 620-626. <http://dx.doi.org/10.1016/j.chemosphere.2012.08.057>
- [15] Kang, J., Ma, T.-F., Zhang, P., *et al.* (2016) Characterization of Soluble Microbial Products in a Drinking Water Biological Aerated Filter. *Environmental Science and Pollution Research*, **23**, 8721-8730. <http://dx.doi.org/10.1007/s11356-015-5973-6>
- [16] Holbrook, R.D., Yen, J.H. and Grizzard, T.J. (2006) Characterizing Natural Organic Material from the Occoquan Watershed (Northern Virginia, US) Using Fluorescence Spectroscopy and PARAFAC. *Science of the Total Environment*, **361**, 249-266. <http://dx.doi.org/10.1016/j.scitotenv.2005.11.020>
- [17] Meng, F., Huang, G., Yang, X., *et al.* (2013) Identifying the Sources and Fate of Anthropogenically Impacted Dissolved Organic Matter (DOM) in Urbanized Rivers. *Water Research*, **47**, 5027-5039. <http://dx.doi.org/10.1016/j.watres.2013.05.043>
- [18] Wu, J., Zhang, H., Shao, L.-M. and He, P.-J. (2012) Fluorescent Characteristics and Metal Binding Properties of Individual Molecular Weight Fractions in Municipal Solid Waste Leachate. *Environmental Pollution*, **162**, 63-71. <http://dx.doi.org/10.1016/j.envpol.2011.10.017>
- [19] Wu, J., Zhang, H., Yao, Q.-S. and He, P.-J. (2012) Toward Understanding the Role of Individual Fluorescent Components in DOM-Metal Binding. *Journal of Hazardous Materials*, **215-216**, 294-301. <http://dx.doi.org/10.1016/j.jhazmat.2012.02.075>
- [20] Kelton, N., Molot, L.A. and Dillon, P.J. (2007) Spectrofluorometric Properties of Dissolved Organic Matter from Central and Southern Ontario Streams and the Influence of Iron and Irradiation. *Water Research*, **41**, 638-646. <http://dx.doi.org/10.1016/j.watres.2006.11.001>
- [21] Riopel, R., Caron, F. and Siemann, S. (2014) Fluorescence Characterization of Natural Organic Matter at a Northern Ontario Wastewater Treatment Plant. *Water Air and Soil Pollution*, **225**, 2126. <http://dx.doi.org/10.1007/s11270-014-2126-3>
- [22] Brunson, C. and Baker, A. (2002) Principal Filter Analysis for Luminescence Excitation-Emission Data. *Geophysical Research Letters*, **29**, 9-1-9-4. <http://dx.doi.org/10.1029/2002GL015977>
- [23] Moberg, L., Robertsson, G. and Karlberg, B. (2001) Spectrofluorimetric Determination of Chlorophylls and Pheopigments Using Parallel Factor Analysis. *Talanta*, **54**, 161-170. [http://dx.doi.org/10.1016/S0039-9140\(00\)00650-0](http://dx.doi.org/10.1016/S0039-9140(00)00650-0)
- [24] Stedmon, C.A., Markager, S. and Bro, R. (2003) Tracing Dissolved Organic Matter in Aquatic Environments Using a New Approach to Fluorescence Spectroscopy. *Marine Chemistry*, **82**, 239-254. [http://dx.doi.org/10.1016/S0304-4203\(03\)00072-0](http://dx.doi.org/10.1016/S0304-4203(03)00072-0)
- [25] Caron, F., Siemann, S. and Riopel, R. (2014) Characterization of the Natural Organic Matter (NOM) in Groundwater Contaminated with ^{60}Co and ^{137}Cs Using Ultrafiltration, Solid Phase Extraction and Fluorescence Analysis. *Journal of Environmental Radioactivity*, **138**, 331-340. <http://dx.doi.org/10.1016/j.jenvrad.2013.12.024>
- [26] Stedmon, C.A. and Markager, S. (2005) Tracing the Production and Degradation of Autochthonous Fractions of Dissolved Organic Matter by Fluorescence Analysis. *Limnology and Oceanography*, **50**, 1415-1426. <http://dx.doi.org/10.4319/lo.2005.50.5.1415>
- [27] Caron, F. and Smith, D.S. (2011) Fluorescence Analysis of Natural Organic Matter Fractionated by Ultrafiltration: Contrasting Between Urban-Impacted Water, and Radio-Contaminated Water from a Near-Pristine Site. *Water Air and Soil Pollution*, **214**, 471-490. <http://dx.doi.org/10.1007/s11270-010-0439-4>
- [28] Cuss, C. and Guéguen, C. (2012) Impacts of Microbial Activity on the Optical and Copper-Binding Properties of Leaf-Litter Leachate. *Frontiers in Microbiology*, **3**, 166. <http://dx.doi.org/10.3389/fmicb.2012.00166>
- [29] Caron, F., Sharp-King, K., Siemann, S. and Smith, D.S. (2010) Fluorescence Characterization of the Natural Organic Matter in Deep Ground Waters from the Canadian Shield, Ontario, Canada. *Journal of Radioanalytical and Nuclear Chemistry*, **286**, 699-705. <http://dx.doi.org/10.1007/s10967-010-0735-x>
- [30] Borraro, V., Riopel, R., Caron, F. and Siemann, S. (2012) Laboratory Study on the Impact of pH and Salinity on the Fluorescence Signal of Natural Organic Matter (NOM) Relevant to Groundwaters from a Canadian Shield Sampling Site. *Water Quality Research Journal of Canada*, **47**, 131-139. <http://dx.doi.org/10.2166/wqrjc.2012.028>
- [31] Burstein, E.A., Vedenkina, N.S. and Ivkova, M.N. (1973) Fluorescence and the Location of Tryptophan Residues in Protein Molecules. *Photochemistry and Photobiology*, **18**, 263-279. <http://dx.doi.org/10.1111/j.1751-1097.1973.tb06422.x>
- [32] Determann, S., Reuter, R., Wagner, P. and Willkomm, R. (1994) Fluorescent Matter in the Eastern Atlantic Ocean. Part 1: Method of Measurement and Near-Surface Distribution. *Deep Sea Research Part A: Oceanographic Research Pa-*

- pers*, **41**, 659-675. [http://dx.doi.org/10.1016/0967-0637\(94\)90048-5](http://dx.doi.org/10.1016/0967-0637(94)90048-5)
- [33] Lakowicz, J.R. (2006) Principles of Fluorescence Spectroscopy. Springer, New York. <http://dx.doi.org/10.1007/978-0-387-46312-4>
- [34] Kumke, M.U. and Frimmel, F.H. (2002) Stationary and Time-Resolved Fluorescence for Humic Substances Characterization. In: Frimmel, F.H., Abbt-Braun, G., Heumann, K.G., et al., Eds., *Refractory Organic Substances in the Environment*, Wiley VCH, Weinheim, 215.
- [35] Jagdale, G.B., Grewal, P.S. and Salminen, S. (2005) Both Heat-Shock and Cold-Shock Influence Trehalose Metabolism in an Entomopathogenic Nematode. *Journal of Parasitology*, **91**, 988-884. <http://dx.doi.org/10.1645/GE-504R.1>
- [36] Kim, Y.H., Han, K.Y., Lee, K. and Lee, J. (2005) Proteome Response of Escherichia Coli Fed-Batch Culture to Temperature Downshift. *Applied Microbiology and Biotechnology*, **68**, 786-793. <http://dx.doi.org/10.1007/s00253-005-0053-3>
- [37] Weber, M.H. and Marahiel, M.A. (2003) Bacterial Cold Shock Responses. *Science Progress*, **86**, 9-75. <http://dx.doi.org/10.3184/003685003783238707>
- [38] Yao, Y., Li, Y., Guo, X., et al. (2016) Changes and Characteristics of Dissolved Organic Matter in a Constructed Wetland System Using Fluorescence Spectroscopy. *Environmental Science and Pollution Research*, **23**, 12237-12245. <http://dx.doi.org/10.1007/s11356-016-6435-5>
- [39] Bridgeman, J., Baker, A., Carliell-Marquet, C. and Carstea, E. (2013) Determination of Changes in Wastewater Quality through a Treatment Works Using Fluorescence Spectroscopy. *Environmental Technology*, **34**, 3069-3077. <http://dx.doi.org/10.1080/09593330.2013.803131>
- [40] Cohen, E., Levy, G.J. and Borisover, M. (2014) Fluorescent Components of Organic Matter in Wastewater: Efficacy and Selectivity of the Water Treatment. *Water Research*, **55**, 323-334. <http://dx.doi.org/10.1016/j.watres.2014.02.040>
- [41] Morel, F.M.M. and Hering, J.G. (1993) Principles and Applications of Aquatic Chemistry. John Wiley & Sons, New York.
- [42] Stevenson, F.J. (1982) Humus Chemistry: Genesis, Composition, Reactions. John Wiley & Sons, New York.
- [43] Ferrari, G.M. and Mingazzini, M. (1995) Synchronous Fluorescence Spectra of Dissolved Organic Matter (DOM) of Algal Origin in Marine Coastal Waters. *Marine Ecology-Progress Series*, **125**, 305-315. <http://dx.doi.org/10.3354/meps125305>
- [44] Kowalczuk, P., Stoń-Egiert, J., Cooper, W.J., Whitehead, R.F. and Durako, M.J. (2005) Characterization of Chromophoric Dissolved Organic Matter (CDOM) in the Baltic Sea by Excitation Emission Matrix Fluorescence Spectroscopy. *Marine Chemistry*, **96**, 273-292. <http://dx.doi.org/10.1016/j.marchem.2005.03.002>



Submit or recommend next manuscript to SCIRP and we will provide best service for you:

Accepting pre-submission inquiries through Email, Facebook, LinkedIn, Twitter, etc.

A wide selection of journals (inclusive of 9 subjects, more than 200 journals)

Providing 24-hour high-quality service

User-friendly online submission system

Fair and swift peer-review system

Efficient typesetting and proofreading procedure

Display of the result of downloads and visits, as well as the number of cited articles

Maximum dissemination of your research work

Submit your manuscript at: <http://papersubmission.scirp.org/>