Eutrophication impacts on characteristics of natural organic matter: a laboratory approach based on *Euglena gracilis* and *Microcystis aeruginosa* cultivation

M. Leloup, R. Nicolau, V. Pallier & G. Feuillade-Cathalifaud
Groupement de Recherche Eau, Sol, Environnement (GRESE), University of Limoges, France

Abstract

Surface water resources can be largely impacted by recurrent seasonal bloom phenomena of algae and cyanobacteria. Algal Organic Matter (AOM) generated presents different composition and properties from that of Natural Organic Matter (NOM). The objective of this work was thus to characterize AOM generated by an alga, *Euglena gracilis*, and a cyanobacteria, *Microcystis aeruginosa*, and to evaluate its impacts on NOM during and after eutrophication. The alga and the cyanobacteria were cultivated in laboratory experiments and the evolution of AOM characteristics during the different growth phases were assessed by organic matter fractionation according to hydrophobicity and Specific UV Absorbance (SUVA) determination. The AOM characteristics were then compared to those of NOM from both an eutrophic pond and a river. The AOM characteristics were both algal species and growth phase dependent. During lag and exponential phases, AOM was hydrophilic and mainly produced by metabolic activity. During stationary and decline phases, the percentages of hydrophobic and transphilic compounds progressively increased because of cell mortality. In natural water, the percentage of hydrophilic compounds reached 10–30% of the DOM. In this study this percentage reached more than 50% for AOM during the decline phase for both species and 45% for NOM of an eutrophic pond during summer, when phytoplanktonic contribution was maximum. The fractionation of OM according to hydrophobicity can thus be used to identify and differentiate the eutrophication origin of OM.
Keywords: eutrophication, organic matter, fractionation, SUVA.

WIT Transactions on Ecology and The Environment, Vol 171, © 2013 WIT Press
www.witpress.com, ISSN 1743-3541 (on-line)
doi:10.2495/WRM130171

1 Introduction

Eutrophication is of major concern since its impacts and consequences on water resources management and drinking water production are serious environmental problematic. It causes disruptions on water ecosystem functioning by modifying food webs (Oliver and Ganf [1]) and degrades water quality by releasing organic material responsible for color, taste and odor (Dokulil and Teubner [2]). Besides, cyanobacteria produce hazardous toxins for health (Hitzfeld *et al.* [3]).

Organic matter fractionation according to the hydrophobic character is commonly used for Natural Organic Matter (NOM) characterization. The protocol allows separating organic compounds into four fractions which characteristics depend on the environmental processes and the origins of the molecules (Thurman [4], Leenheer and Croué [5]). Wang *et al.* [6] showed that organic molecules from allochthonous origin (mainly soil and catchment inputs) are more hydrophobic with high molecular weight. On the contrary, organic molecules from autochthonous contribution (phytoplankton and bacteria) are more hydrophilic with low molecular weight. The repartition of DOM in natural waters thus depends on the degree of humification and the contribution of the different sources.

Algal Organic Matter (AOM) is produced by algae and cyanobacteria and it represents a large part of the autochthonous origin of NOM. Several authors already studied Algal Organic Matter properties. These studies were limited to the exponential and stationary growth phases and the results obtained depended on the growth conditions (culture medium or light intensity), the considered growth phase and the cultivated species. However, whatever the experimental conditions, the produced organic matter was mainly composed of hydrophilic compounds, this fraction representing 57 to 71% of the AOM (Henderson *et al.* [7], Li *et al.* [8], Zhang *et al.* [9]). Nowadays, few studies deal with the fate of AOM after bloom has collapsed and the impacts of AOM inputs on NOM characteristics in the long term still needs to be determined. NOM from water resources not affected by eutrophication is generally composed of 10–30% of hydrophilic compounds (Thurman [4], Labanowski and Feuillade [10]). Recurrent inputs of AOM may modify the distribution of the NOM fractions. It is thus important to understand the way water resources can be enriched in organic matter during and after bloom phenomenon in order to predict changes in NOM properties and adapt water treatment processes.

The objectives of this work were first to quantify and characterize organic matter produced by algae and cyanobacteria during life cycle in laboratory controlled conditions, and second to determine the impacts of blooms on the characteristics of NOM from an eutrophic pond. AOM and NOM were characterized by organic matter fractionation according to hydrophobicity and Specific UV Absorbance (SUVA) index.

2 Materials and methods

2.1 Alga and cyanobacteria cultivation

Two species commonly found in natural waters – one alga, *Euglena gracilis* (*E.g*) and one cyanobacteria, *Microcystis aeruginosa* (*M.a*) – were cultivated in laboratory conditions. The analyses protocols and the culture conditions are described in Leloup *et al.* [11].

The two species were cultivated in 1L erlenmayer flasks filled up with 500mL of synthetic Chu 10 modified medium. During the growth period (lag, exponential and the beginning of the stationary phases) two flasks were sacrificed every week to determine cell density and AOM characteristics. All the analyses were performed in duplicate from samples grown in separate occasions. During the late stationary phase and the decline phase the frequency of the analysis was reduced. The cultivation lasted 112 days under 30µmol.photon. $m^{-2}.s^{-1}$ illumination with a 15h/9h light/dark cycle at 23 ± 1°C. 20 erlenmayer flasks inoculated at the same time were required per studied species.

The cell density and the growth phases were determined by flow cytometry (FACS Calibur, Becton Dickinson). AOM was quantified by Dissolved Organic carbon (DOC) measurements by using a Shimadzu TOC-L carbon analyser and characterized by both fractionation according to hydrophobicity and SUVA index.

2.2 Study site and sampling strategy

Pigeard Pond is an artificial shallow eutrophic pond (max. depth 2.30m, area 3.2 ha) located at latitude 45°54'N and longitude 1°11'E in the Limousin area, France (Figure 1). The pond is surrounded by fields and supports recreational fishing activities. Two sampling points were chosen to study the impacts of phytoplanktonic blooms on the characteristics of NOM. The first one is located at the Valette River (V), just before its entrance into the pond (Figure 1). During the study period, the river was not affected by blooms phenomenon. At this sampling point the river is about 30cm depth and 40cm wide. The second sampling point is located about 30cm under the water surface of Pigeard pond (P) (Figure 1). One sampling was performed per season at each sampling point.

2.3 Analyses performed

Global parameters (Temperature ± 0.1°C, pH ± 0.2, Dissolved Oxygen (DO) ± 0.2 mgO_2/L) were measured *in-situ* at each sampling point with a multi-parameters MS5 probe (OTT).

Chlorophyll *a* (Chl *a*) was determined according to the French Standard NF T 90-117. Chl *a* was extracted in acetone 90% (v/v) and concentration was calculated according to the Lorenzen equation. Analyses were performed in triplicate.

The DOC analyses were realised in duplicate by a Shimadzu TOC-L analyzer (precision ± 50µgC/L) according to the Non-Purgeable Organic Carbon measurement procedure.

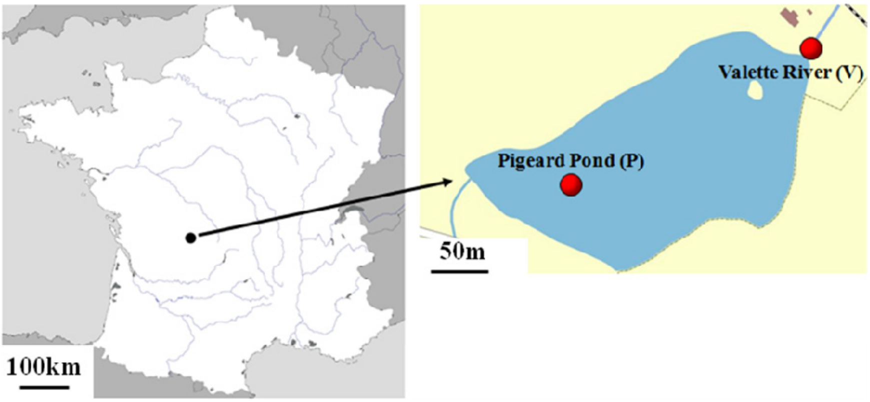


Figure 1: Location of the study site in the Limousin area, France (left) and sampling points (right).

XAD fractionation was performed in duplicate. Humines and particulate organic matter were first removed from samples by filtration on 0.45µm nitrate cellulose filters. Acidification of samples at pH 2 with HCl 37% followed by filtration on 0.45µm membranes allowed separating Humic Acids (HA). 306mL of samples were then passed through DAX-8 and XAD-4 resins in series. The Hydrophobic compounds (HPO) were adsorbed on DAX-8 resin, the Transphilic compounds (TPH) on XAD-4 resin and the Hydrophilic compounds (HPI) were not adsorbed on any resin. A partition coefficient k' of 50 was chosen for this study as advised by Labanowski and Feuillade [12] for the study of hydrophilic compounds. The filtration flow and the resins volumes were respectively fixed at $50mL.h^{-1}$ and 5mL. The concentrations of each fraction were determined by DOC measurements. HA were not considered in the repartition because of their poor contribution to DOC.

The Specific UV absorbance (SUVA index) is defined as the ratio of the UV absorbance to the DOC concentrations. It allows estimating the aromaticity of organic molecules and correlates with their hydrophobic character (Weishaar *et al.* [13], Croué [14]). The SUVA index was calculated for the global sample and for each organic matter fraction. The UV absorbance at 254nm was measured by using a Shimadzu PharmaSpec 1700 spectrophotometer (precision ± $0.005cm^{-1}$) with 1cm-long quartz cells.

3 Results and discussion

3.1 Natural waters characteristics

The evolution of the global parameters of water samples from the two sampling points is given Table 1. The river water parameters did not evolve significantly over the one year study period unlike those of the pond water. Pigeard Pond water was influenced by the weather conditions and the proliferations of cyanobacteria that occurred from spring to autumn, with a maximum of Chl *a* during summer. During this period DOC increased substantially with inputs of AOM. pH and DO values were also high as a consequence of eutrophication.

Table 1: Evolution of the global parameters of Pigeard Pond (P) and Valette River (V) in function of seasons.

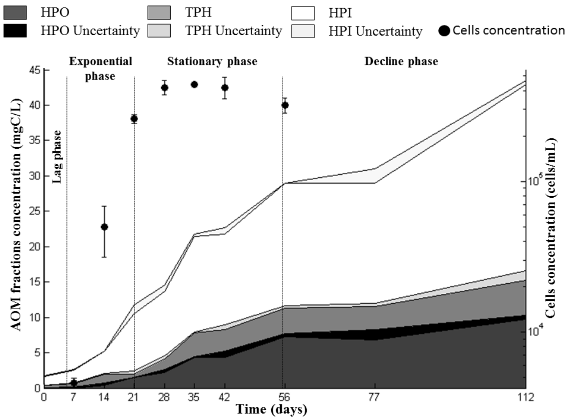
Parameters	**Winter**		**Spring**		**Summer**		**Autumn**	
	P	**V**	**P**	**V**	**P**	**V**	**P**	**V**
Temperature (°C) ± 0.1°C	6.5	7.5	19.9	13.3	21.0	13.0	9.3	9.4
pH ± 0.2	7.6	7.1	10.0	7.1	9.8	7.2	7.5	6.9
DO (mgO_2/L) ± 0.2 mgO_2/L	12.3	10.3	12.4	10.0	13.4	7.8	7.8	9.2
Chl *a* (µg/L)	54 ±3.0	14 ±2.0	90 ±16	43 ±6.0	169 ±28.0	31 ±11	103 ±18.0	47 ±8.0
DOC (mgC/L) ± 50µgC/L	3.75	3.55	6.00	5.05	6.16	4.37	5.09	6.06

3.2 AOM impacts on NOM characteristics

3.2.1 Evolution of AOM fractionation

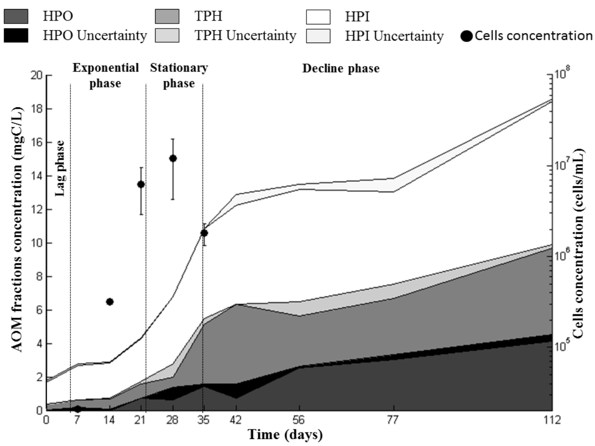
The evolution of the concentrations of AOM fractions in function of growth phases for *E. gracilis* and *M. aeruginosa* is given Figures 2 and 3 respectively. A same evolution can be observed for both the alga and the cyanobacteria. During the lag phase, little DOC was produced and it was only composed of HPI. During the exponential phase, DOC increased and the produced AOM was mainly composed of HPI (increased by 7.41 and 1.18 mgC/L for the alga and the cyanobacteria respectively). During the stationary phase, the HPI concentration still increased as the ones of HPO (6.19 mgC/L for *E.g* and 0.88 mgC/L for *M.a*) and TPH (3.09 and 2.84 mgC/L for *E.g* and *M.a* respectively). During the decline phase, HPI was still actively produced for both species whereas HPO and TPH increased to a lesser extent than during the stationary phase and this increase is species dependent. The quantity of AOM produced and its composition depended on the growth phase and on the specie. Indeed, *E. gracilis* produced more AOM than *M. aeruginosa* (at the end of the experiment DOC concentration reached almost 45 mgC/L for *E.g* and 19 mgC/L for *M.a*) despite cells concentrations were lower for the alga.

The percentages of each organic matter fraction produced by *E. gracilis* and *M. aeruginosa* were dependent of the specie and the growth phase (Figure 4). As expected from Figures 2 and 3, a same evolution of AOM characteristics can be observed for the two species. In accordance with Henderson *et al.* [7], Li *et al.* [8] and Zhang *et al.* [9], AOM produced by both the alga and the cyanobacteria



Parameter	Standard deviation Min.-Max.
Cells per mL	400 - 67,900
HPO (mgC/L)	0.04 - 1.40
TPH (mgC/L)	0.03 - 1.40
HPI (mgC/L)	0.02 - 1.97

Figure 2: Evolution of HPO, TPH and HPI concentrations produced by *E. gracilis* in function of growth phases.



Parameter	Standard deviation Min.-Max.
Cells per mL	700 - 7,700,000
HPO (mgC/L)	0.04 - 0.82
TPH (mgC/L)	0.03 - 0.84
HPI (mgC/L)	0.03 - 0.82

Figure 3: Evolution of HPO, TPH and HPI concentrations produced by *M. aeruginosa* in function of growth phases.

was mainly hydrophilic during the lag and exponential phases with 70 to 80% of HPI. HPI is thus mainly produced by metabolic activity because mortality is low during these two phases. The part of hydrophobic and transphilic compounds increased from the late exponential phase to the advanced decline phase and reached then 24% for HPO and between 14 and 29% for TPH. During decline phase the part of HPI only reached 45 to 60% but HPI remained the major fraction.

The hydrophobic character of AOM increased during the stationary and the decline phases probably because HPO and TPH compounds were from intracellular compounds released by cell lyses and particulate organic matter from cells fragments generated by photo-dissolution and leaching processes (Leloup *et al.* [11]). A difference in AOM composition between the two species can be observed from stationary phase (Figure 4). AOM produced by *M. aeruginosa* was more transphilic and less hydrophilic than AOM generated by the alga.

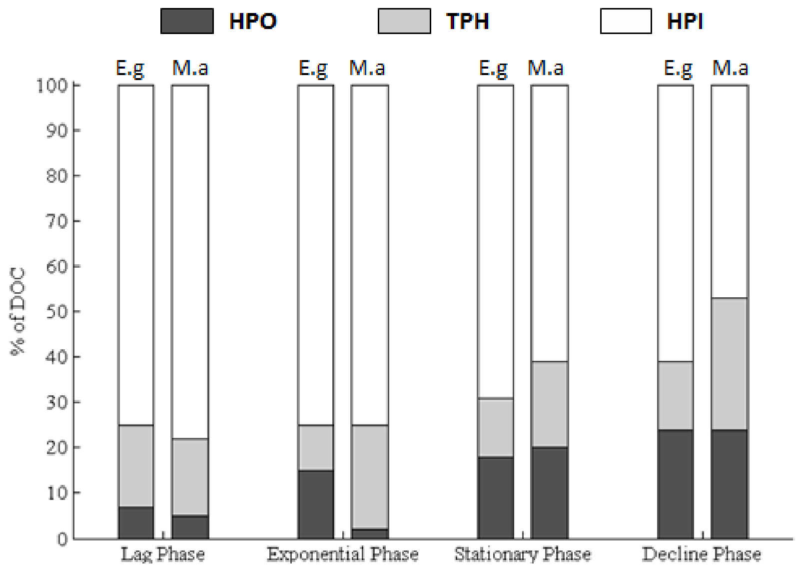


Figure 4: Evolution of the AOM fractions percentages of *E. gracilis* (*E.g*) and *M.aeruginosa* (*M.a*) during life cycle (± 3%).

3.2.2 AOM impacts on NOM fractionation

AOM characteristics were very different from that of NOM. Indeed, NOM is usually composed of 20-30% of hydrophilic compounds (Labanowski and Feuillade [10]) whereas this fraction reached 45 to 80% in AOM depending on the specie and the growth phase.

The evolution of the organic matter fractions in water samples from Pigeard Pond and Valette River in function of seasons is given Figure 5. The characteristics of organic matter from Valette River did not vary significantly over the year. During winter and autumn the repartition of organic fractions was quite the same for the two water resources which shows that over these seasons the characteristics of NOM in the pond are mainly influenced by the river's inputs. In both cases, maximum HPO percentage was reached during autumn probably because the rainfall brought many organic compounds from the catchment and also because of the decline of the phytoplanktonic population. Moreover, the contributions from allochthonous sources rich in hydrophobic compounds (particularly leaves' supply and degradation) are more important during autumn.

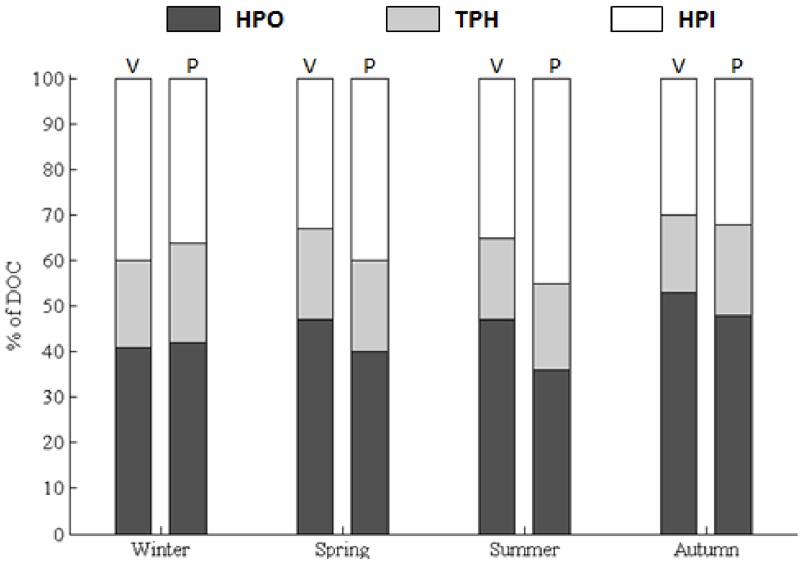


Figure 5: Evolution of the organic matter fractions percentages of Pigeard Pond (P) and Valette River (V) according to the seasons (± 3%).

However the characteristics of NOM from Pigeard Pond varied substantially during spring and summer with an increase of the hydrophilic part. The difference between the two sampling points was the most important during summer, when phytoplankton population was the highest (maximum Chl *a*) in the pond. This increase in HPI percentage can be explained by inputs of AOM of high hydrophilic content compared to NOM. Besides, the DOC content of Pigeard Pond during this period increased by 2.41mgC/L (Table 1) when compared to the values obtained in winter. DOC inputs during spring and summer is mainly linked to phytoplankton proliferation. During this period, phytoplankton produces an additional DOC load when compared to winter. The phytoplankton thus modifies the characteristics of NOM by increasing its HPI content. When compared to the values in winter, during summer in the pond, Chl *a* increased three times (Table 1) and HPI percentage by 10%, reaching near 45% of DOC.

3.3 Evolution of SUVA index during and after bloom phenomena

3.3.1 Determination of AOM SUVA index

A comparison between global SUVA index and SUVA index of organic matter fractions of AOM and NOM from Glane River is given Table 2. Whatever the growth phase, global SUVA index of AOM produced by the alga and the cyanobacteria was lower than that of NOM. This result correlated well with the higher HPI content of AOM when compared to NOM. SUVA indexes of the HPO fractions were approximately the same whatever the origin of the organic matter. The SUVA indexes of the HPO fraction of AOM increased between stationary and decline phases unlike TPH and HPI fractions. However, TPH of *M.a* decreased in a larger extent than that of *E.g* while HPI of *E.g* decreased in a larger extent than that of *M.a*. Global SUVA index of phytoplanktonic origin decreased but that of *E.g* in a larger extent than that of *M.a*. So AOM characteristics are highly influenced by TPH and above all by HPI fractions.

The SUVA indexes of HPI from *E.g* and TPH from *M.a* were very low when compared to the SUVA indexes of same fractions of different origins. So these two fractions exhibited unusual characteristics and are very atypical.

Table 2: Comparison of SUVA index of AOM (Leloup *et al.* [11]) and NOM (Labanowski and Feuillade [10]).

Origin		Global SUVA index ($L.cm^{-1}.gC^{-1}$)	Fractions SUVA index ($L.cm^{-1}.gC^{-1}$)		
			HPO	TPH	HPI
E. g	Stationary phase	13.9 ± 0.1	19.5 ± 1.6	17.1 ± 1.4	8.4 ± 0.6
	Decline phase	11.2 ± 0.1	26.4 ± 3.9	13.5 ± 1.4	6.9 ± 0.1
M. a	Stationary phase	10.7 ± 1.3	12.0 ± 0.4	7.3 ± 0.6	11.9 ± 0.5
	Decline phase	10.4 ± 0.8	19.0 ± 2.7	4.0 ± 1.0	11.6 ± 0.4
Glane River		18.3 ± 0.2	21.4 ± 0.2	16.2 ± 0.2	12.7 ± 0.2

3.3.2 Comparison of SUVA indexes of NOM impacted by AOM

The evolution of global SUVA index and SUVA indexes of the HPI fractions of Pigeard Pond and Valette River is presented Figure 6. SUVA index values of Valette River were higher than that of Pigeard Pond whatever the sampling period. This can be explained by a more intensive phytoplanktonic activity generating higher inputs of AOM of low aromatic character in the pond when compared to the river.

Moreover, global SUVA index and SUVA index of the HPI fractions decreased from spring to summer and increased from autumn to winter whatever the sampling point. As Weishaar *et al.* [13] and Croué [14] showed that SUVA index represents the aromaticity and the hydrophobicity of organic molecules, decreasing values of global SUVA during spring and summer well correlated with the increasing HPI content in the pond. The lower SUVA values of the pond compared to that of the river were also well correlated with the higher HPI content observed in the pond. Indeed, in the pond, inputs of AOM of high hydrophilic content and low global and HPI SUVA index can explain these results. However, no significant variation of HPI content and no algal development were observed in the river during this period. So decreasing values of SUVA in the river did not result from phytoplanktonic activity. This can be explained by low contribution of organic matter from land, which is more complex and aromatic than autochthonous organic matter. Indeed rainfalls are low during spring and summer compared to autumn and winter and the part of organic matter brought by runoff decreases while that from microbial activity increases. Because SUVA of organic matter produced by microbial activity is lower than that of terrestrial origin, global SUVA index of the river decreased.

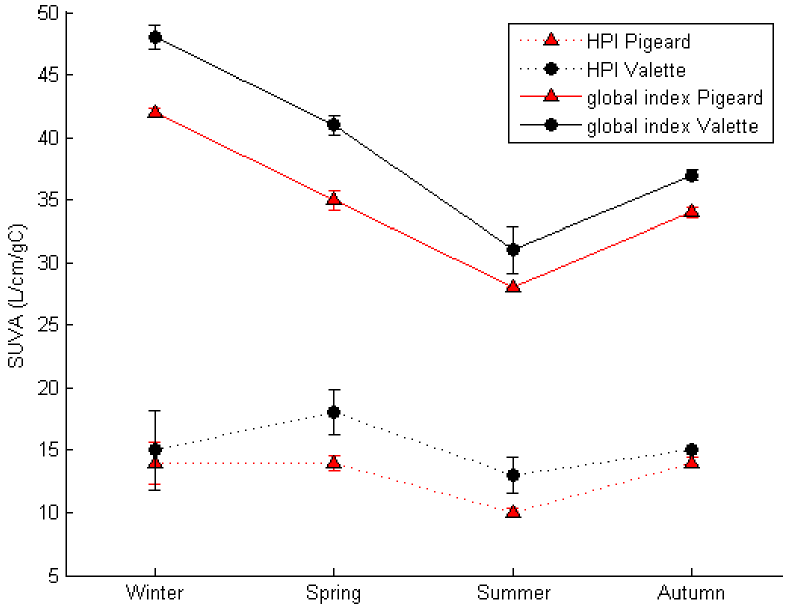


Figure 6: Evolution of global SUVA index and SUVA of the HPI fractions of Pigeard Pond and Valette River.

4 Conclusions

Under laboratory cultivation, the quantity of AOM produced by an alga and a cyanobacteria and its characteristics depended on both the considered specie and the growth phase. However the evolution of AOM characteristics was similar for both species and AOM was mainly hydrophilic whatever the specie and the growth phase.

During lag and exponential phases, AOM produced was mainly hydrophilic and represented almost 70% of the dissolved organic matter. The part of hydrophobic and transphilic compounds increased from the late exponential phase to the advanced decline phase and reached then 24% for HPO and between 14 and 29% for TPH. The production of such organic molecules correlated with cell death and photo-degradation of particulate organic matter. Besides, global SUVA index of AOM was low and particularly the SUVA index of the HPI fraction, when compared to those of natural waters. The monitoring of organic matter characteristics from Pigeard Pond and Valette River during the study period underlined that NOM was particularly affected by AOM inputs during spring and summer. The percentages of HPI in NOM increased during this period in the eutrophic Pigeard pond and reached 45% of DOC during summer, *i.e.* 10% higher than during winter, period during which phytoplanktonic activity is low. The repartition of NOM in the river did not vary significantly because the contribution from phytoplankton was low compared to the one of the pond. The global SUVA index for the pond was lower than the one of the river although both decreased during spring and summer. The inputs of algal organic compounds with low aromaticity and hydrophobicity decreased the global SUVA index for the pond as the SUVA index of the HPI fraction.

So the eutrophication phenomenon influenced the characteristics of NOM by increasing the hydrophilic fraction and decreasing both global SUVA index and SUVA index of the HPI fraction. However these modifications of NOM properties may depend on the ability of the water resource to auto-regulate and on the importance of the bloom phenomenon.

References

[1] Oliver, R.L. and Ganf, G.G., Freshwater blooms. In: *The Ecology of Cyanobacteria*. Whitton, B.A. and Potts. M., (eds). Dordrecht: Kluwer Academic Publishers. pp. 149-194, 2000.

[2] Dokulil, M.T. and Teubner, K., Cyanobacterial dominance in lakes. *Hydrobiologia*, **438**, pp. 1-12, 2000.

[3] Hitzfeld, B.C., Höger, S.J., and Dietrich, D.R., Cyanobacterial Toxins: Removal during Drinking Water Treatment, and Human Risk Assessment. *Environmental Health Perspectives*, **108(1)**, pp. 113-122, 2000.

[4] Thurman, E.M., Developments in Biochemistry: Organic Geochemistry of Natural Waters. Nijhoff, M. and Junk, W. (eds). Dordrecht, 1985.

[5] Leenheer, J.A. and Croué, J-P., Characterizing aquatic dissolved organic matter. *Environmental Science and Technology*, **37(1)**, pp. 16A-26, 2003.

[6] Wang, L., Wu, F., Zhang, R., Li, W. and Liao, H., Characterization of dissolved organic matter fractions from Lake Hongfeng, Southwestern China Plateau. *Journal of Environmental Sciences*, **21(5)**, pp. 581-588, 2009.

[7] Henderson, R.K., Baker, A., Parsons, S.A. and Jefferson, B., Characterisation of algogenic organic matter extracted from cyanobacteria, green algae and diatoms. *Water Research*, **42(13)**, pp. 3435-3445, 2008.

[8] Li, L., Gao, N., Deng, Y., Yao, J. and Zhang, K., Characterization of intracellular and extracellular algae organic matters (AOM) of Microcystic aeruginosa and formation of AOM-associated disinfection byproducts and odor and taste compounds. *Water Research*, **46(4)**, pp. 1233-1240, 2012.

[9] Zhang, Y., Tian, J., Nan, J., Gao, S., Liang, H., Wang, M. and Li, G., Effect of PAC addition on immersed ultrafiltration for the treatment of algal-rich water. *Journal of Hazardous Materials*, **186(2-3)**, pp. 1415-1424, 2011.

[10] Labanowski, J. and Feuillade, G., Combination of biodegradable organic matter quantification and XAD-fractionation as effective working parameter for the study of biodegradability in environmental and anthropic samples. *Chemosphere*, **74(4)**, pp. 605-611, 2009.

[11] Leloup, M., Nicolau, R., Pallier, V., Yéprémian, C. and Feuillade-Cathalifaud, G., Organic matter produced by algae and cyanobacteria: quantitative and qualitative characterization. *Journal of Environmental Sciences*. Under correction, 2013.
[12] Labanowski, J. and Feuillade, G., Dissolved organic matter: Precautions for the study of hydrophilic substances using XAD resins. *Water Research*, **45(1)**, pp. 315-327, 2011.
[13] Weishaar, J.L., Aiken, G.R., Bergamaschi, B.A., Fram, M.S., Fujii, R. and Mopper, K., Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environmental Science and Technology*, **37(20)**, pp. 4702-4708, 2003.
[14] Croué J-P., Isolation of humic and non-humic NOM fractions: Structural characterizations. *Environmental Monitoring and Assessment*, **92(1-3)**, pp. 193-207, 2004.