



## Urban lakes: interaction between phytoplankton dynamics and trace metal speciation

Viet Tran Khac, Brigitte Vinçon-Leite, Gilles Varrault, Bruno Lemaire, Bruno Tassin, Nilo Nascimento

### ► To cite this version:

Viet Tran Khac, Brigitte Vinçon-Leite, Gilles Varrault, Bruno Lemaire, Bruno Tassin, et al.. Urban lakes: interaction between phytoplankton dynamics and trace metal speciation. Daniel Thevenot. 12th edition of the World Wide Workshop for Young Environmental Scientists (WWW-YES-2012) - Urban waters: resource or risks?, May 2012, Arcueil, France. HAL-ENPC, WWW-YES-2012 (13), 2012, WWW-YES. <hal-00709659>

**HAL Id: hal-00709659**

**<https://hal-enpc.archives-ouvertes.fr/hal-00709659>**

Submitted on 19 Jun 2012

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



# Urban lakes: interaction between phytoplankton dynamics and trace metal speciation

Viet TRAN KHAC\*, Brigitte VINÇON-LEITE\*, Gilles VARRAULT\*, Bruno Jacques LEMAIRE\*, Bruno TASSIN\* and Nilo NASCIMENTO\*\*

\* Laboratoire Eau Environnement Systèmes Urbains (LEESU), Ecoles des ponts ParisTech, 6-8 Av. Blaise Pascal, 77455 Champs-sur-Marne, France.  
(E-mail: [tran-khv@leesu.enpc.fr](mailto:tran-khv@leesu.enpc.fr))

\*\* Federal University of Minas Gerais, Hydraulic and water resource engineering department, av. Antônio Carlos, 6627, 31270-901, Belo Horizonte, MG, Brazil.

## Abstract

Urban lakes have a particular influence on the water cycle in urban catchments. Thermal stratification and a longer residence time of the water within the lake can boost the phytoplankton production. On the other hand, trace metals are naturally found in environment in trace amounts due to erosion and physic-chemical or biological alteration processes. Trace metals are essential to growth and reproduction of organisms. However, they are also well known for their toxic effects on animals and humans. Their ecotoxicity depends on metal properties and chemical speciation (particulate, dissolved: labile or bioavailable and inert fractions). The interaction between phytoplankton production and trace metal speciation is not well understood until present time. The aim of this research is twofold: (1) to analyze the driving processes of the phytoplankton production in an urban lake through physical-chemical and biological field data collection and (2) simultaneously to survey the chemical speciation of trace metals in this lake. To address these issues, a field survey and laboratory analysis, including physical-chemical, biological variables, organic matter and trace metal concentrations, have been designed and implemented in an urban study site: Lake Créteil in France

## Keywords

Urban lakes; trace metal; phytoplankton; transparent exopolymer particles; speciation

## INTRODUCTION

Urban lakes are different from rural and natural lakes: they are specific elements of storm water drainage networks, highly artificial, small and shallow water bodies. These water bodies, such as ponds and grave-pits lakes, are common in urban and peri-urban landscape. Urban lakes play a key role in recreation and storing rainwater (Stephen Birch, 1999). According to Downing et al. (2006), 99,2% of water bodies in the world is 10ha or less. These aquatic ecosystems are highly impacted by watershed urbanization and by urban activities such as industry, traffic waste and drainage systems (Friese et al., 2010). Moreover, they are also very sensitive to a large variety of planetary environmental changes (PECs) such as increased CO<sub>2</sub>, increased air temperature and atmospheric pollution (Poff *et al.*, 2002). Despite the abundance and the usefulness of these water bodies for societies, we notice a lack of research on these urban lakes in comparison with large and deep lakes (Jeppesen 1998, Moss 2010, Davies et al. 2008).

Because urban lakes are often small and shallow, they should respond rapidly and strongly to climatic fluctuations (Whitehead *et al.* 2007). Climate change could increase wet periods during winter and spring and increase drought during summer time. Consequently, water residence time would be modified (Bates *et al.* 2008). During extreme climatic events, rain runoff effluents lead to a decline of bacteriological and chemical water quality and thus impacting the sanitary state

and the functioning of aquatic ecosystems (Characklis *et al.* 2005, Patz *et al.* 2005, Allan & Castilla 2007). Heat wave events can enhance phytoplankton production rates (Nõges 2009, Moijj *et al.* 2005). Urban lakes are generally polymictic lakes and they may be particularly vulnerable to changes in the water temperature and the mixing patterns caused by climatic extremes (Wagner & Adrian 2009).

All these environmental and anthropic disturbances can modify both hydrodynamic and physical-chemical conditions of urban lakes, which can favor phytoplankton development or even reinforce eutrophication due to higher nutrient internal cycling and better light climate (Nõges 2009, Bertolo *et al.* 1999 ).

### **Scientific context of the research**

Two sources of trace metals exist in aquatic environment: natural source and anthropic source. Trace metals are naturally present in environment due to erosion and physical-chemical or biological alteration processes. Human activities represent an anthropic source in aquatic ecosystem. Trace metals enter urban water bodies via several pathways such as atmospheric deposition, industrial and domestic wastewater discharge, agricultural activities or storm water runoff (Thévenot *et al.*, 2009).

Trace metals are vital components for growth, development and reproduction of living organisms (Buffle, 1988; Tessier *et al.* Turner, 1995). A lack of metals can be a limited factor for development of organism, for example a lack of iron can cause a diminution of phytoplankton development (Martin *et al.* Fitzwater, 1988; Martin *et al.*, 1991). However, a high concentration of metals can be extremely toxic. Indeed, trace metal toxicity for aquatic organisms have been known for a long time and are considered as a serious threat for human and animal health (Bryan and Langston, 1992).

Ecotoxicity and bioavailability of trace metal are not directly related to total metal concentration but depends on the form and the nature of chemical species (Florence, 1986, Scoullou and Pavlidou, 1997, Lores and Pennock, 1998). In aquatic systems, trace metals can exist in many different chemical forms, which determine bioavailability and toxicity to aquatic organisms (Hirose, 2006, Morel *et al.*, 2003). Trace metals can be particulate or dissolved (free or complexed to organic and inorganic ligands). Under different meteorological and physical-chemical conditions (pH, salinity, hardness of water, organic carbon content...), trace metal form can change (Forstner and Wittmann 1979). In dissolved metal fraction, metal can be labile (free or weakly linked to organic or inorganic ligand) or inert (no interaction with surrounding environment). Labile metal is defined as free metal or weakly linked to mineral ions (hydroxyl, carbonate) or to organic molecules (Thevenot, Meybeck *et al.* 2002). Labile metal is also defined as “easily modifiable, exchangeable by easy and rapid interaction with surrounding solution” (Tusseu-Vuillemin 2005). In other word, labile metal is constituted of cationic free form or weakly associated to mineral ions (Thevenot, Meybeck *et al.* 2002). Because of their mobility in aquatic environment, this fraction is directly related to bioavailability of metal. These labile metals, which can be easily linked to aquatic organisms, are a good indicator of toxicity. Therefore, it is important to study the speciation of trace metal in aquatic systems.

So far, we know that dissolved organic matter (DOM) plays a key role on trace metal speciation in aquatic systems. DOM can be divided into 2 categories: the humic substances and the non-humic substances. According to Thurman (1985), humic substances are majority, up to 70% of DOM in comparison with non humic substances, estimated at 25%. Humic and fulvic acids are the most studied because they play a key role on complexing free metal ion in most of the aquatic systems. However, in urban areas, the non-humic, mainly hydrophilic DOM, can

constitute a significant fraction as a result of treated or untreated wastewater discharge (Pernet-Coudrier et al., 2011). The binding ability of wastewater organic matter has been highlighted during an acute toxicity test of copper with *Daphnia Magna* (Pernet-Coudrier et al., 2008). In aquatic ecosystems, non-humic DOM can also be produced by microbiological activity, mainly phytoplankton and bacteria. The complexing ability of trace-metal of this natural aquagenic DOM, called extracellular polymeric substances (EPS) is still unclear.

Our research will focus on one type of EPS, called Transparent Exopolymer Particles (TEP). TEP are defined as particles formed from acid polysaccharides. During phytoplankton blooms in freshwater and marine systems, phytoplankton and bacteria abundantly exude TEPs. Diatoms are especially known for producing TEPs during their growth (Passow, U. 2002). TEPs can play a role on trace metal speciation because of the large affinity of trace metal to surface-active exopolymers (Tye, Jespen, & Lick, 1996). Between 40-90% of trace elements can be adsorbed by the TEPs in marine system (Passow, U. 2002). However, until now, the TEPs have been more widely studied in marine systems than in freshwaters. Moreover, very few results exist about the TEP characteristics in urban lakes.

In this context, the main question of our research is: How are the TEPs generated by phytoplankton and bacteria during blooms events influencing the trace-metal speciation in an urban lake?

To achieve this issue, monthly field campaigns aimed at measuring physical-chemical and biological variables and trace metal concentrations have been implemented in Lake Créteil (France). Physical parameters (temperature, pH, conductivity, oxygen, photosynthesis active radiations (PAR)) are monitored in situ with probes. Laboratory analysis (total phosphorus, phosphates, major cations and anions, TEP...) are performed on water samples. In this paper, the organization of the field campaigns and the laboratory analysis will be presented. The first results will be exposed and discussed.

A second study site (not be presented in this paper) with different climate and socio-economic context has been selected, Lake Pampulha (Brazil). Lake Créteil survey takes part of the research project PULSE (Peri-Urban Lakes, Society and Environment) supported by ANR (National Research Agency). Lake Pampulha survey takes part of the research project MAPLU (Storm water management project, funded by the Brazilian Agency for Funding Study and Projects – FINEP).

## **MATERIAL & METHODS**

In order to investigate the interactions between phytoplankton and trace metals, we have designed a field survey aimed at monitoring the main environmental parameters driving the phytoplankton dynamics, the transparent exopolymer particles production which could consequently influence the metal speciation.

The field survey includes high-frequency measurements of water temperature aimed at providing continuous time series at 3 depths, giving access to the stratification and mixing patterns of the water column. Water temperature is an essential parameter, which directly, through algal growth rate, plays a key role in phytoplankton production. Moreover, mixing or stratification of the water column will control in a different way the growth of the algal species and will lead to different algal succession. These high-frequency measurements will allow us to understand at small time scale the physical control of phytoplankton dynamics.

The field campaigns also include, at a regular frequency, (1) vertical profiles of physical parameters aimed at describing the phytoplankton growth conditions: temperature, pH, conductivity, photosynthesis active radiations (PAR); (2) vertical profiles of parameters associated to the phytoplankton biomass: Chlorophyll a produced by 4 main phytoplankton groups and oxygen concentrations; (3) water sampling for further analysis of nutrients and TEPs concentrations and (4) specific water sampling and field filtration for trace metal speciation analysis .

More details on the field and the analysis protocols are given in a following paragraph.

### Description of study site

Lake Créteil is located in Ile-de-France region (48°46'N 02°27'E), in an urbanized zone at 15km from Paris. The lake originates from an excavation of alluvial sediments near the confluence of the Rivers Seine and Marne. Its surface is 40.8 ha, the volume is 1.5 106 m<sup>3</sup>, the maximum depth 6m and the mean depth 4.5 m. The lake is mainly supplied by groundwater but also supplied by a stormwater outlet of the urbanized Mont-Mesly area. This lake is used for recreational activities (sailing, windsurfing, fishing...) and plays an important role in providing quality of life for the inhabitants of this highly urbanized area.

Lake Créteil is polymictic with a density stratification lasting from days to weeks during the summer, which impacts the primary production.

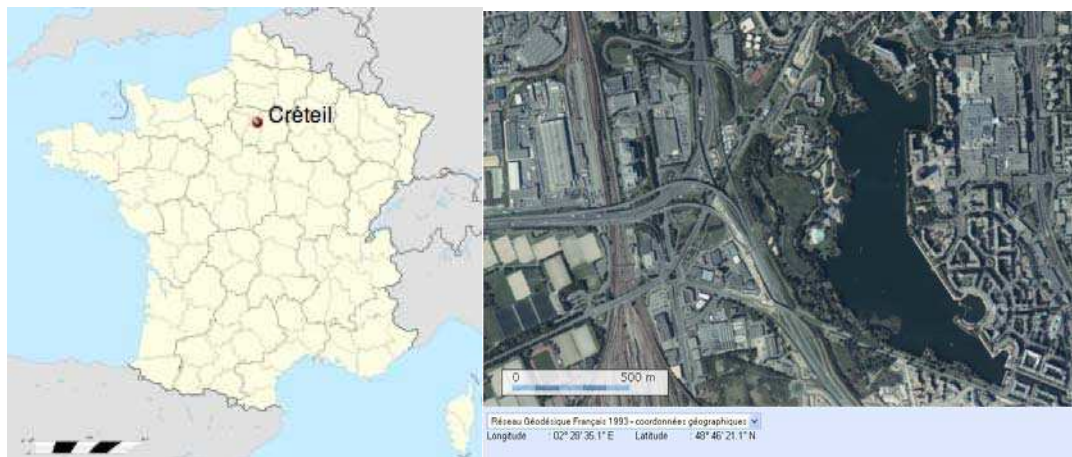


Figure 1: Lake Créteil location (Créteil, France) (www.geoportail.fr)

### Continuous temperature monitoring

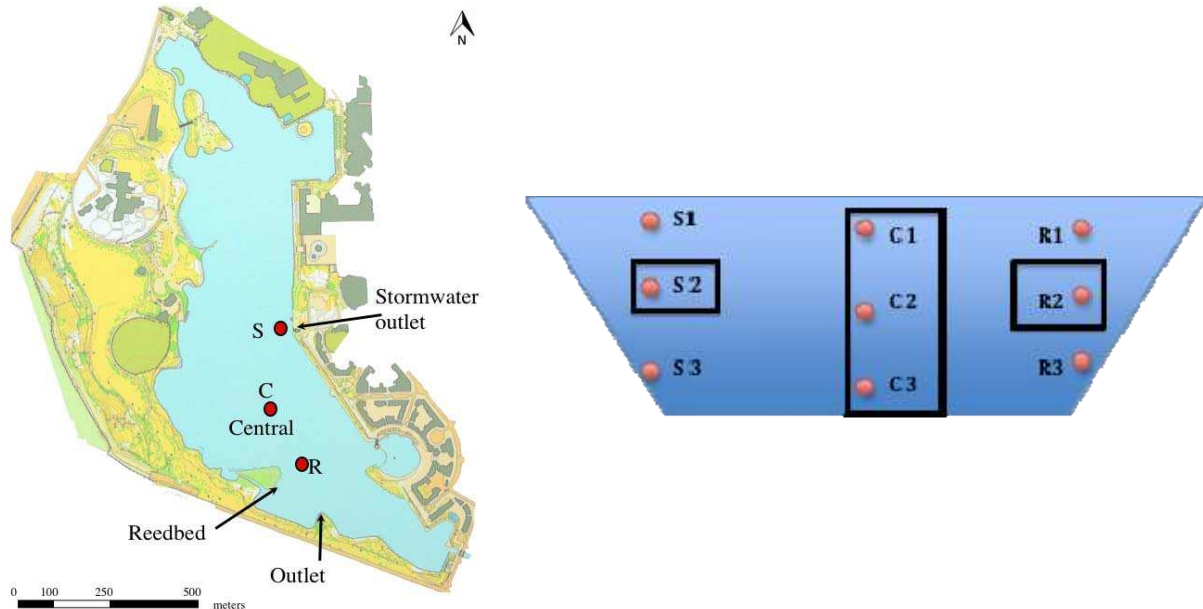
At the central point C (see Figure 2), three temperature sensors installed at three fixed depths (0.55m, 0.94 m and 3.62m) will measure continuously the water temperature. The measurement time step is 30mn. This sensor chain has been working since August 2011.

### The monthly field campaigns

The monitoring campaigns, conducted on Lake Créteil, include three sampling points located on an horizontal transect, from the stormwater outlet to the lake outlet : the « S » point is close to the stormwater outlet, the « R » point is located next to a rich organic reedbed area and the central point « C », is located between S and R (Figure 2). For each sampling point, three depths are defined: surface (0.5 meters), middle (1.5 meters) and bottom (3.5 meters). For each campaign, a “lake average sample”, named “M” is mixed from 9 samples collected at three

depths at the three points (S1, S2, S3, C1, C2, C3, R1, R2, R3). Water samples are collected using a NISKIN bottle.

For each campaign, 6 samples are collected for trace metal and TEP analysis: S2 (middle of the water column at point S), C1, C2, C3 (3 depths at central point), R2 (middle of the water column at point R) and M (lake average point).



**Figure 2: Location of the three sampling points on the lake Créteil (Left) and sampling depth (black rectangle) for laboratory analysis**

### Probe measurements

Vertical profiles of physical (salinity, temperature, oxygen, conductivity, photosynthetically active radiation (PAR), water transparency) and biological (chlorophyll a) parameters are also monitored *in situ* using probes: CTD Seabird43, LI-COR193 (underwater PAR sensor), Fluoroprobe BBE.

### Water sampling

#### *Trace-metals*

Trace metal analysis suffers of very high contamination risks. In order to avoid these risks, all materials used are in high-density polyethylene and have been prepared in a clean room. These materials have been washed according to a specific procedure: first washing cycle in detergent solution EXTRA at 5% prepared with reverse osmosis water during 24h and second washing cycle in nitric acid solution at 5% prepared with ultrapure water.

For each field campaign, 3 metal fractions are analyzed: (1) total metals, (2) total dissolved metals and (3) inert metals.

Total metal, analyzed in the raw water sample (50mL), corresponds to particulate and dissolved metal fractions. Total dissolved metal is operationally defined as the filtrate of 0.45-micron filtration. The sample volume is 20mL. Inert metals are determined using chelating disks where a chelating resin retains labile metal. The chelating disk (EMPORE™) contains a chelating resin “Chelex 100”, constituted of styrene-divinylbenzene co-polymer containing imino-diacetic acid groups. Imino-diacetic acid groups have a strong affinity toward transition metal that allows fixing them by a coordinate bond. Inert metals go through the chelating disk. The sample volume for inert metals is 20mL. The labile metal fraction is quantified by difference between total dissolved metal and inert metal.

The concentrations of the 3 metal fractions mentioned above are determined using inductively coupled plasma mass spectrometry (ICP-MS). Trace metals determined are: Al, Cr, Mn, Fe, Ni, Cu, Zn, Cd, Pb, Ti, V, Co, Ag.

#### *Transparent exopolymer particles (TEP)*

Transparent exopolymer particles (TEP) are operationally defined as particles retained on polycarbonate filters (0.45 $\mu$ m), which stains with the cationic dye alcian blue. The dyeing blue alcian solution 8GX, which can stain both sulfated and carboxylated polysaccharides, is prepared at concentration 0.02% m/v with 0.06% acetic acid to maintain pH at 2.5 (Passow & Alldredge, 1995b).

TEP concentration is measured using Passow and Alldredge's method (1995). 100 mL of raw water are filtered through 0.45 $\mu$ m pore size polycarbonate filters and stained with alcian blue. Stained filters are rinsed with ultrapure water to remove excess blue alcian and keep in frozen until analysis. TEP stained by blue alcian are re-dissolved in 6mL of 80% sulphuric acid for 3h. Absorption at 787nm was measured spectrophotometrically. Measures are conducted in triplicate. Average concentration will be expressed as a standardized Xanthan equivalent in  $\mu$ g.l<sup>-1</sup> ( $\mu$ gXeq.l<sup>-1</sup>).

#### Laboratory analysis

Water samples are analyzed in laboratory to determine nutrient (total phosphorus, phosphate, nitrate) and major anion and cation concentration. Total Phosphorus and phosphate analysis are measured according to AFNOR norm. Total Phosphorus is mineralized with persulfate and phosphate is colored by ascorbic acid. Absorption at 880nm is measured with a spectrophotometer. Nitrate concentration is determined by ionic chromatography.

## **PRELIMINARY RESULTS**

Our preliminary results allow us to present the seasonal physical-chemical and biological patterns of Lake Creteil recorded from August 2011 to March 2012.

First results of metal concentration by ICP-MS were obtained very recently (30<sup>th</sup> March 2012) and the data treatment is under progress. We are still working on the validation of these results.

TEP analysis planned for monthly field campaigns will start in May 2012. Presently, we are conducting laboratory tests in order to validate our final protocol.

Conductivity is very high in Lake Creteil; it was nearly constant at about 1600  $\mu$ S/cm between October 2011 and March 2012. This value corresponds to historical data of the lake. Lake Créteil is rich on chloride and sulfate, which contribute to a high conductivity (Bonneau et al. 2005) pH is about 7.8 between October 2011 and March 2012.

Between August 2011 and March 2012, the surface temperature at the central point C was maximal at 23°C in 05-August 2011. Temperature profile was homogenous in the water column from December 2011 to March 2012. The temperature was minimal (1.6°C) in February 2012, when the lake was frozen. The temperature gradients recorded in the lake during the summer 2011 are low, around 0.4°/m.



## Sulfate and chloride

Ionic chromatography analysis confirmed that sulfate and chloride are the major anions in the lake. The concentration of these anions in the lake is largely higher in comparison to the others. Sulfate and chloride concentrations are respectively about 450 mg/L and 150 mg/L.

## Phosphorus total and phosphate

**Table 1 Total Phosphorus and phosphate concentrations for February and March 2012**

Points	February		March	
	Phosphate (µg/L)	Total Phosphorus (µg/L)	Phosphate (µg/L)	Total Phosphorus (µg/L)
M	9.1	36.5	2.5	137.4
S2	21.2	52.6	2.8	32.3
R2	8.2	305.8	2.5	56.7
C1	29.9	65.6	5.5	34.1
C2	9.0	35.2	1.7	33.3
C3	8.2	36.6	5.6	35.2

We have to notice that in February, air temperature was negative during 2 weeks. The lake was frozen during the field campaign. It was not possible to reach the regular sampling points and we had to conduct measurements close to point position. It can explain why there was an extremely high phosphorus total concentration at the reedbed point R2. Phosphate concentration seems to be more homogenous for March than February.

## Chlorophyll a

Between October 2011 and March 2012 chlorophyll a concentration measured by Fluoroprobe BBE varies from 5 – 20 µg/L.

## CONCLUSION AND PERSPECTIVES

In order to investigate interactions between trace metal and phytoplankton, we suppose that organic matter at the lake central point C is essentially produced by phytoplankton. On the contrary, organic matter from storm water effluent will be mostly of urban origin and the organic matter in the reedbed area will have a more humic structure. Comparing and analyzing the data set of these 3 points will allow us to disentangle the influence of phytoplankton on trace metal speciation.

Monitoring of physical parameters and laboratory analysis will be useful to understand hydrodynamical and environmental conditions of the lake. In a second step, we intend to synthesize the knowledge of the lake functioning in an hydrodynamical and biological model, which can simulate and explain phytoplankton development in the lake. Understanding of phytoplankton dynamic helps us to understand better TEP production, which can explain trace metal speciation and bioavailability.

## REFERENCES

- Allan D.J., and Castillo M.M., 2007. Stream ecology: structure and function of running waters. Springer, Dordrecht, the Netherlands, pp. 436.
- Bates, B.C., Z.W. Kundzewicz, S. Wu & J.P. Palutikof (Eds.). 2008: Climate Change and Water. Technical Paper of the Intergovernmental Panel on Climate Change, IPCC Secretariat, Geneva, 210 pp.
- Bonneau E., Pelletier-Creusot S., Brechet O., Chassany V., 2008. "Qualité du lac de Créteil de 2001 à 2004", Rapport DSEA Val de Marne.
- Bryan G W, Langston W J, 1992. Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: A review. *Environmental Pollution*, 76(2): 89–131.
- Buffle, J. (1988). "Complexation reactions in aquatic systems, an analytical approach " Ellis Horwood series in analytical chemistry: 304-380.
- Characklis G.W., Dilts M.J, Simmons O.D., Likirdopulos, C.A., Krometis, L.A. and Sobsey M.D., 2005. Microbial Partitioning to Settleable Particles in Stormwater. *Water Research*, 39 : 1773-1782.
- Florence, T.M. (1986), Electrochemical approaches to trace element speciation in waters, *Analyst*, 111, 489-505.
- Forstner, U. and G. T. W. Wittmann (1979). "Metal Pollution in the Aquatic Environment. Springer- Verlag." New York: 289 p.
- Hirose, K. Chemical speciation of trace metals in seawater: a review. *Anal. Sci.* 2006, 22, 1055–1063.
- Lores, E. and Pennock, J. (1998), The effect of salinity on binding of Cd, Cr, Cu and Zn to dissolved organic matter, *Chemosphere*, 37, 861-874.
- Louis, Y., G. Varrault, et al. (2009). "Détermination des concentrations en métaux labiles et dissous dans la Marne et ses affluents durant un épisode de temps de pluie." Programme PIREN-Seine.
- Martin J. H. and Fitzwater S. E. (1988). "Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic". *Nature* 331 (6154), 341-343.
- Martin J. H., Gordon R. M. and Fitzwater S. E. (1991). "The case for iron". *Limnology and Oceanography* 36 (8), 1793-1802.
- Mooij, W.M., S. Hülsmann, L.N. De Senerpont Domis, B.A. Nolet, P.L.E. Bodelier, P.C.M. Boers, L.M. Dionisio Pires, H.J. Gons, B.W. Ibelings, R. Noordhuis, R. Portielje, K. Wolfstein & E.H.R.R. Lammens. 2005. The impact of climate change on lakes in the Netherlands: a review. *Aquatic Ecology*, 39: 381-400.
- Morel, F. M. M.; Price, N. M. The biogeochemical cycles of trace metals in the oceans. *Science* 2003, 300, 944–947
- Nöges, T. 2009. Relationships between morphometry, geographic location and water quality parameters of European lakes. *Hydrobiologia*, 633: 33-43.
- Patz J.A., Campbell-Lendrum D., Holloway T. and Foley J.A., 2005. Impact of regional climate change on human health. *Nature*, 438: 310-317.
- Pernet-Coudrier, B., 2008. Influence de la matière organique dissoute sur la spéciation et la biodisponibilité des métaux: cas de la Seine, un milieu sous forte pression urbaine LEESU. Université Paris Est Créteil Val de Marne, Créteil, pp. 292.
- Pernet-Coudrier, B., Varrault, G., Saad, M., Croue, J.P., Dignac, M.F., Mouchel, J.-M., 2011a. Characterisation of dissolved organic matter in Parisian urban aquatic systems: predominance of hydrophilic and proteinaceous structures. *Biogeochemistry*.
- Tessier, A., D. R. Turner, et al. (1995). "Metal speciation and bioavailability in aquatic systems." John Wiley & sons, Chichester: 679p.
- Thévenot D.R., Lestel L., Tusseau-Vuillemin M.H., Gonzalez J.L, Meybeck M. 2009. « Les métaux dans le bassin de la Seine » – Programme Interdisciplinaire de Recherche sur l’Environnement de la Seine (PIREN-SEINE)
- Thevenot, D., M. Meybeck, et al. (2002). "Métaux lourds: des bilans en mutation, Piren-Seine, Rapport de synthèse 1998-2001."

- Thurman. 1985. Organic geochemistry of natural waters. Dordrecht: Kluwer Academic Publishers Group.
- Tushara Chaminda, G. G., F. Nakajima, et al. (2009). "Zn and Cu complexation with DOM in wastewater treatment plant effluent." 19ème congrès international de la SETAC.
- Tye, R., Jepsen, R., & Lick, W. (1996). Effects of colloids, flocculation, particle size, and organic matter on the adsorption of hexachlorobenzene to sediments. *Environmental Toxicology and Chemistry*, 15, 643–651.
- Wagner, C. & R. Adrian. 2009. Cyanobacteria dominance: Quantifying the effects of climate change. *Limnology and Oceanography*, 54: 2460-2468.
- Whitehead, P.G., R.L. Wilby, R.W. Battarbee, M. Kernan & A.J.Wade. 2009. A review of the potential impacts of climate change on surface water quality. *Hydrological Sciences*, 54: 101-123.

*13-WWW-YES-2012-Tran-Khac-Paper-2012-06-15.doc*