Electron-optical characterization of nano- and micro-particles in raw and treated waters; an overview

Gary G. Leppard*, Denis Mavrocordatos & Didier Perret*****

* Aquatic Ecosystem Management Research Branch, National Water Research Institute, 867 Lakeshore Road, P.O. Box 5050, Burlington, Ontario, Canada L7R 4A6. Gary.Leppard@ec.gc.ca
** EAWAG, Swiss Federal Institute for Environmental Science and Technology, Postfach 611, Uberlandstrasse 133, CH-8600 Dübendorf, Switzerland. Denis.Mavrocordatos@eawag.ch
*** EPFL, Swiss Federal Institute of Technology, Laboratory of Soil Science, CH-1015 Lausanne, Switzerland. Didier.Perret@epfl.ch

Abstract

State-of-the-art information is presented on the analysis, by transmission electron microscopy (TEM), of aquatic colloidal particles in the size range of 3 to 500 nm least dimension, with a focus on nano-particles (1-100 nm). Case studies include selections from both natural waters and waters undergoing treatment. The "species" of nano-particles receiving the greatest attention are: humic substances, polysaccharide fibrils, hydrous iron oxides, viruses, clay minerals, refractory cell debris, and heavy metal agglomerates on biological surfaces. Artifacts and how to both detect and minimize them are outlined. Correlative use of TEM with other imaging techniques is emphasized, along with associated spectroscopy. Noted is the potential of computerized image analysis for quantifying colloids on a "per colloid species" basis, using water samples centrifuged onto electron microscope grids.

Keywords Analytical microscopy; characterization methodology; nano-particles; natural waters; transmission electron microscopy; treated waters

Introduction

The characteristics of aquatic nano- and micro-particles, in the colloidal size range, must become better known in order to understand their behaviour in water and consequently to (1) model their natural activities and to (2) design more cost-effective treatment facilities. Aquatic colloids provide a nano-scale milieu into and onto which dissolved chemicals (e.g., contaminants, nutrients) can escape from the bulk aqueous solution, without significantly affecting via gravitational settling the vertical movement of the colloid-chemical association (Gustafsson and Gschwend, 1997). At the lower end of the submicron scale, massive numbers of fine colloids and suspended macromolecules are likely to influence water quality and particle separation processes.

Conventional transmission electron microscopy (TEM) is an essential technique for the analysis of these fine colloids, yielding descriptions of size, shape and internal differentiation on a "per colloid basis". The descriptions can be supplemented to permit characterization of individual fine colloid "species", and heterogeneous aggregates of them, when TEM is used in conjunction with accessory techniques (Buffle et al., 1998; Leppard and Buffle, 1998). The major accessory techniques, applicable to pre-selected individual colloids, are: energy dispersive spectroscopy (EDS); selected-area electron diffraction (SAED); electron energy-loss spectroscopy (EELS); energy filtered TEM (EF-TEM); and electron-opaque selective probes. Used correlatively in parallel (and with the ever-improving atomic force microscopy, or AFM), these techniques provide a powerful suite of analytical electron microscopies (AEM) for studying environmental particles (Wilkinson et al., 1999; Mavrocordatos et al., 2000; Taillefert et al., 2000; Webb et al., 2000). Currently, the adaptation of TEM for the characterization of colloidal particles is progressing well (Perret et al., 1991, 1994; Leppard, 1992; Lienemann et al., 1998; Jackson and Leppard, 2002), and the new information can be supplemented by synchrotron data (on the atomic environment of selected elements) obtained from scanning transmission X-ray microscopy, or STXM (Lawrence et al., 2003). STXM represents a promising new frontier; it exploits the fact that soft X-rays interact with almost all elements to allow mapping of chemical species based on bonding structure. STXM uses near-edge X-ray absorption spectroscopy (NEXAFS) as its contrast mechanism, with soft X-rays providing a spatial resolution of better than 50 nm. Through the application of tunable soft X-rays and appropriate analysis of X-ray absorption spectra, in the form of NEXAFS image sequences, quantitative chemical mapping at a spatial scale below 50 nm is achievable (Jacobsen et al., 2000).

Correlated multi-method interdisciplinary approaches to water analysis, which include AEM and which use independent methods to provide chemical, biological and environmental context for electron-optical observations, are increasingly likely to yield practical information on both nano- and micro-particle activities (Perret et al., 1994; Droppo et al., 1997; Jackson et al., 1999; Mavrocordatos et al., 2000; Taillefert et al., 2000; Webb et al., 2000; Lawrence et al., 2003; Leppard et al., 2003). Confocal laser scanning microscopy (CLSM), used correlatively with TEM and STXM, is contributing to this multi-method interdisciplinary approach (Lawrence et al., 2003).

New nano-scale observations should contribute to our understanding of environmental cycling of chemicals (Gustaffson and Gschwend, 1997; Mavrocordatos et al., 2000; Taillefert et al., 2000), aquatic aggregation processes (Buffle et al., 1998; Liao et al., 2002), the interactions of natural organic matter with aquatic organisms (Campbell et al., 1997) and heavy metal deposition from solution onto microbial cell surfaces (Webb et al., 2000; Chatellier et al., 2001; Jackson and Leppard, 2002). Electron-optical analyses which correlate morphological data with data from both physical and molecular probes will facilitate further development of colloid "speciation" at the nano-scale. Such nano-scale data is already leading to improved characterizations of contaminant transport agents in surface waters (Leppard et al., 1998; Taillefert et al., 2000; Webb et al., 2000) and also of colloidal promoters of biofouling in membrane filters used for water purification (Liao et al., 2003). For heterogeneous aggregation processes, new speciation information should allow one to pinpoint which colloids are the major contributors to (1) engineered aggregation processes, (2) contaminant binding in treatment facilities, (3) biofouling of immersed surfaces and (4) the transfer of toxic chemicals to environmental sinks. This overview on the environmental application of AEM focuses on recent advances in our understanding of aquatic nano-particles through the application of TEM-based methods.

Selected case studies relating particle characterization to contaminant transport

In 1997, Campbell et al. used a multi-method approach with TEM to demonstrate the accumulation of nano-particles of natural organic matter on the surfaces of living cells (a chlorophyte, a cyanobacterium and cells isolated from fish gills). Using environmentally-relevant pH values and organic matter concentrations, they produced data consistent with the idea that the nano-particles might exert a direct effect on organism physiology at the interface between a living cell and its aquatic environment. Campbell et al. (1997) then considered the implications of their findings for the interactions of toxic solutes with aquatic biota.

In 1998, Leppard et al. isolated micro-particles and flocs from the surface water of Hamilton Harbor (ON, Canada) by size class (in ranges running from <0.02 μm to >80 μm), and then analyzed each size class for polycyclic aromatic hydrocarbons (PAH) which contaminate the harbor. They found with TEM that the micro-particles and flocs consisted mainly of aggregated colloids, among them numerous nano-particles of minerals (clays, iron oxyhydroxides, manganese-rich colloids, biogenic silica) and of organics (cell debris, fibrils and humic substances). Heterogeneous porous flocs larger than 20 μm accounted for 98% of phenanthrene binding, 89% of fluoranthene binding and 85% of pyrene binding.

In 2000, Mavrocordatos et al. used AEM in an interdisciplinary approach to characterize individual colloids in successive compartments of a complex peat-karst-spring ecosystem (Vallée-des-Ponts, Switzerland). Their results revealed that humic-rich globules and colloidal iron formed intimate entities which then underwent specific physico-chemical transformations during their transport through a karstic aquifer, leading to final entities with drastically different characteristics. The investigation focused on the nano-scale characteristics of reactive particulate entities upstream and downstream from the karstic aquifer. The authors point out that their analytical approach should have major consequences for estimating the vulnerability of karstic aquifers with regard to pollution events.

Also in 2000, Taillefert et al. employed a suite of analytical techniques with TEM to study the chemical speciation of iron and lead in the water column of a lake characterized by a biogenic meromixis (Paul Lake, MI, USA). The primary goal was to determine the effect of hydrous iron oxides and natural organic matter on the speciation of lead, so as to understand better the cycling of lead in a natural lacustrine ecosystem. The results indicated that specific suspended nano-particles (hydrous iron oxides and organic fibrils) aggregated together to become complex micro-particles which then became enriched in lead; such a process would not be described accurately by surface adsorption models. Previously, Lienemann et al. (1997a) showed that sub-micrometre manganese-rich crusts produced by Mn-oxidizing bacteria, at the oxic-anoxic interface of this highly stratified lake, had selectively pre-concentrated cobalt ions with respect to the water column. As the Mn-rich and Fe-rich layers were partially overlapping at depth, classical bulk chemical analyses of the particulate material had to be supplemented by TEM-EDS measurements at the "per particle" level to yield unambiguous insights into the selective scavenging of Pb by $\mathrm{Fe}_{\mathrm{part}}$ and of Co by $\mathrm{Mn}_{\mathrm{part}}$. Their approach, although time-consuming, opened new perspectives for the documentation of the speciation of toxic trace metals at the solid-solution interface.

Selected case studies relating particle characterization to water treatment problems

In 1996, Liss et al. used TEM and SCLM in parallel to describe the nano- and micro-scale architecture of engineered flocs sampled from a (pulp and paper mill) oxygenated activated sludge effluent system (Thunder Bay, ON, Canada). At a practical resolution of 0.001 μm, they described bacteria and other colloid-sized particles embedded in a complex matrix of extracellular polymeric substances (EPS). This matrix was rich in 4-6 nm (least diameter) fibrils of EPS which (i) acted as bridges between the diverse colloidal particles within a floc, and which (ii) formed the tenuous boundaries of an extensive intra-floc pore structure. Liao et al. (2002) used a variety of physico-chemical techniques, in association with TEM, to analyze interparticle interactions affecting the stability of sludge flocs sampled from laboratory-scale sequencing batch reactors. With a focus on the three-dimensional arrangement and packing of fibrils, they proposed a conceptual model of floc architecture which relates EPS nano-particles to floc stability in engineered water treatment systems. Since this model was proposed, an interdisciplinary AEM study of contaminated wastewater flocs has demonstrated that different, nano-scale, floc colloids immobilize different heavy metals differentially (Leppard et al., 2003). This finding has implications for heavy metal recovery (and re-use) from activated sludge.

Nano-particles receiving attention; selected microscopy publications

An informative list would include the following:

Humic substances (Myneni et al., 1999; Senesi, 1999; Wilkinson et al., 1999; Mondi et al., 2002), *Polysaccharide fibrils* (Leppard, 1995, 1997; Lienemann et al., 1997b, 1998; Wilkinson et al., 1999), *Hydrous iron oxides* (Perret et al., 2000), *Viruses* (Borsheim et al., 1990; Weinbauer and Peduzzi, 1994), *Clay minerals* (Couture et al., 1996; Jackson and Leppard, 2002), *Refractory cell debris* (Leppard et al., 1997), *Heavy metal agglomerates on cell surfaces* (Webb et al., 2000; Chatellier et al., 2001).

Because of the current great interest in fibrils and hydrous iron oxides, the following studies are noteworthy. Nano-particle associations with other nano-particles must be better understood. Careful specimen preparation of fragile aquatic samples may unravel the complexity of the three-dimensional interactions between fibrils and other biotic or abiotic entities, such as the abundant ones prevailing in marine snow (Heissenberger et al., 1996; Lienemann et al., 1998) and in wastewater flocs (Liss et al., 1996; Liao et al., 2002; Leppard et al., 2003). When the weak electron opacity of organic matter is an obstacle to its identification, without the use of metal-rich markers, nano-scale fibrils can still be visualized by means of EF-TEM at the carbon K-edge (Lienemann et al., 1997b). In contrast, the polysaccharidic nature of EPS fibrils can be ascertained unambiguously by reaction with a specific probe (Ag-proteinate; Lienemann, 1997).

Perret et al. (2000) have demonstrated that hydrous iron oxides, ubiquitous in most freshwaters, exhibit specific morphotypes whose structure is mainly driven (i) by the type of macromolecular organic matter present in the water, and (ii) by the relative concentrations of total iron and natural organic matter (NOM). In the presence of aquagenic EPS, which can form 3-D networks of fibrils, Fe-NOM associations tend to lead to iron oxide nano-granules attached to the surfaces of fibrils, in particular when the ionic strength of the milieu is high and the Fe_{tot}: NOM ratio is low. In contrast, ill-defined intimately-blended Fe-NOM mixtures will be favored at lower ionic strength and high Fe_{tot}:NOM ratio. In the presence of terrestrial humic/fulvic-rich material, the final Fe-NOM entities are spherical globules made of a C-rich core surrounded by amorphous hydrous iron oxides. It has also been shown by Lienemann et al. (1999) that the lacustrine oxidation of Fe(II) may lead to the formation of hydrous iron oxides which stoichiometrically include phosphates, and that this nutrient scavenging naturally acts as an efficient barrier against upward diffusion of phosphates in the water column of Lake Lugano (CH), a stratified yet eutrophic lake. In the case of Fe-humics entities formed during the oxygenation of acidic, anoxic Fe(II)-rich peat waters (Mondi et al., 2002), it has been shown by high resolution TEM-PEELS measurements on individual 100 nm globules that the abrupt rise in pH, O_2, and I in the drainage waters leads to the formation of a compact outer crust of ca. 2-4 nm amorphous Fe-Ca-rich granules at the surface of shrunken humic spheroids, the shape of the latter being dictated mostly by pH and I constraints.

Micro-particles as natural aggregates of nano-particles

Many (or most) of the micro-particles found in surface waters (riverine, lacustrine, marine) and in biological wastewater treatment systems are heterogeneous aggregates of microbes and nano-particles (Droppo et al., 1996; Liss et al., 1996; Leppard et al., 1997, 1998, 2003; Webb et al., 2000; Liao et al., 2002). A generalized description of aquatic colloidal interactions (leading potentially to the formation of micro-particles) has been published by Buffle et al. (1998), who demonstrated that the concentration of stable colloids in a given aquatic ecosystem will depend on the relative proportions of three general classes of native colloids (compact inorganics; large rigid biopolymers; fulvics and fulvic-like substances). For biota-rich flocs, Liao et al. (2002) have proposed a conceptual model of aggregate structure whereby the gel-like matrix consists of two physically distinct regions that are defined by the arrangement of nano-scale EPS which cross-connect individual cells of the microbiota. The physically distinct regions are likely to be differentially affected by agents applied to manipulate floc integrity. Heterogeneity in the packing of (and in the specific chemical composition of) EPS, as revealed by AEM and selective extraction methods, appear to reflect floc stability. For marine biota-rich flocs, Heissenberger et al. (1996) have suggested a scheme (based on TEM observations) for the growth and development of marine snow, and the sometimes subsequent development of economically-undesirable mucilage phenomena (Leppard, 1995). Heissenberger et al. (1996) related the growth of suspended marine snow flocs to (i) the secretion of nano-scale fibrils by microorganism communities and to (ii) many environmental processes which modulate the cross-linking activities of fibrils and the natural activities of the developing flocs.

Artifact detection and minimization in the AEM analysis of colloids

The detection, assessment and minimization of artifacts in the preparation of native aquatic colloids for AEM analyses are described in detail in Leppard and Buffle (1998). The artifacts include those created prior to analysis (e.g., sample mishandling at time of sampling, unnecessary fractionation, perturbations induced by storage, exposure to perturbing chemical agents, excessive pre-concentration). Leppard and Buffle (1998) recommend (i) avoiding sample storage, (ii) minimizing the number of steps in water sample preparation. (iii) paying exquisite attention to detail in the use of any fractionation procedure deemed necessary, and (iv) adapting to particle size polydispersity.

Recommended protocols for obtaining ultrathin sections and optimally-presented whole mounts are provided in Perret et al. (1991), Liss et al. (1996) and Lienemann et al. (1998); for readily-deformed, water-rich flocs, the protocol of Droppo et al. (1996) is recommended. The principle artifacts to consider in the production of sections and whole mounts for analysis are those of dehydration and shrinkage, with extraction being a potential problem in the case of sections; these artifacts are well understood and readily minimized for many kinds of samples. For an orientation with regard to AEM apparatus (TEM, STEM-EDS, EELS, EF-TEM) and rationales for the selection of representative images, consult Mavrocordatos et al. (2000) and Leppard et al. (2003).

Correlative use of TEM with AFM and STXM

Correlated TEM and AFM analyses of aquatic nano-particles (of humic substances and polysaccharides) are described in Wilkinson et al. (1999) while correlated TEM, CLSM and STXM analyses (of colloidal extracellular polymeric substances within a microbial biofilm) are described in Lawrence et al. (2003). The respective goals were to better understand (i) the structure of nano-particle aggregates and (ii) the three-dimensional disposition of major classes of organic macromolecules in the colloidal matrix structure of a microbe-rich aggregate.

Image analysis

To quantify individual native colloid "species" (as defined by a combination of size, compositional and morphological criteria), a practical electron-optical technology is available now (Lienemann et al., 1998), to be used in combination with the quantitative ultracentrifugation of colloids onto TEM grids. User-friendly image analysis has become available (Leppard and Arsenault, 2003) to facilitate TEM-based colloid quantification efforts.

Image analysis has become a powerful technique to extract quantitative data from microscope-based information. In terms of morphology, many parameters are available to allow one to extract valuable data on environmental particles. With a representative specimen, size distributions of nano-scale particles from a heterogeneous suspension, not measurable with bulk techniques, can be quantified with sound statistics. Such measurements can be evaluated with respect to bulk measurements when a population of particles is homogeneous. Recent advances in the analysis of many kinds of particles are relevant to the image analysis of aquatic nano- and micro-particles.

The accurate determination of the particle size distribution of colloidal entities may yield information on the processes driving their behaviour in the milieu (e.g. formation, dissolution, coagulation, sedimentation). For instance, Couture et al. (1996) performed image analysis of ca. 9000 individual colloids collected under state-of-the-art conditions from the deep groundwater of a potential site for repository of nuclear wastes in Switzerland (Wellenberg, LU). It was demonstrated that clays (dominant particle type; chlorite, illite, smectite; ca. 30-4000nm in mean size) were subject to elimination from the waters by orthokinetic (fluid shear-induced) coagulation (slope of the particle size distribution expressed as a Pareto power law: $b = -2.2$).

Mavrocordatos et al. (2002) calculated the size distribution of wood combustion particles. 1200 particles were analyzed and the correlation with a Scanning Mobility Particle Sizer (SMPS) was highly comparable. Recently, Kaegi (2003) traced the source of various atmospheric particles according to their shape. Particles emitted from the gas exhaust of cars were drastically different than the ones coming from the erosion of a tunnel wall. Not-yet published data (results from the authors) show that using ultracentrifugation and EFTEM allows one to classify particles according to their size and composition. Such information is only obtainable from a combination of microscope techniques and image analysis. As well, the regime of aggregation of particles can be determined. The fractal dimension (Df) of an aggregate measured with image analysis informs us how the aggregation took place. For Lin et al (1989) and Jullien (1992), a Df~1.8 corresponds to an aggregation controlled by Brownian motion (Diffusion Limited Colloid Aggregation, DLCA), whereas, when Df ~ 2.1, electrostatic repulsive forces are the driving forces for aggregation (Reaction Limited Colloid Aggregation, RLCA). Such calculations have been used by Fatin-Rouge et al. (2000) to determine the aggregation mechanism of nano-particles analyzed by TEM. In the same manner, Mavrocordatos (2002) showed that the Df of combustion particles could be analyzed by AFM. Results were comparable with previous work on combustion particles.

Conclusion

The above-mentioned selection of applications of analytical electron microscopy in water science exemplifies the progresses performed during the last decade in the development of highly efficient characterization techniques at the micro- and nanometer scale.

Out of its infancy, correlative AEM becomes part of an integrated characterization process of raw or treated waters, from initial sampling to final quantitative results. At every step of the complete process biases have been identified and overcome, while quantitative and representative specimens preparation schemes have been developed; the most suitable microscopies have been pinpointed and optimized with respect to the specificities of the samples and finally, owing to the power of image analysis softwares, representative and quantitative morphological and compositional parameters can be obtained.

Nowadays, microscopic measurements are no longer merely illustrative of suspended particulate in waters but profitably complement results obtained by conventional bulk measurements.

References

Borsheim, K.Y., Bratbak, G. and Heldal, M. (1990). Enumeration and biomass estimation of planktonic bacteria and viruses by transmission electron microscopy. *Appl. Environ. Microbiol.,* **56**, 352-356.

Buffle, J., Wilkinson, K.J., Stoll, S., Filella, M. and Zhang, J. (1998). A generalized description of aquatic colloidal interactions: the three-colloidal component approach. *Environ. Sci. Technol.,* **32**, 2887-2899.

Campbell, P.G.C., Twiss, M.R. and Wilkinson, K.J. (1997). Accumulation of natural organic matter on the surfaces of living cells: implications for the interaction of toxic solutes with aquatic biota. *Can. J. Fish. Aquat. Sci.,* **54**, 2543-2554.

Chatellier, X., Fortin, D., West, M.M., Leppard, G.G. and Ferris, F.G. (2001). Effect of the presence of bacterial surfaces during the synthesis of Fe oxides by oxidation of ferrous ions.*European J. Mineral*., **13**, 705-714.

Couture, C., Lienemann, C.-P., Mavrocordatos, D. and Perret, D. (1996). New directions towards the understanding of physico-chemical processes in aquatic systems. *Chimia*, **50**, 625-629.

Droppo, I.G., Flannigan, D.T., Leppard, G.G., Jaskot, C. and Liss, S.N. (1996). Floc stabilization for multiple microscopic techniques. *Appl. Environ. Microbiol*., **62**, 3508-3515.

Droppo, I.G., Leppard, G.G., Flannigan, D.T. and Liss, S.N. (1997). The freshwater floc: a functional relationship of water and organic and inorganic floc constituents affecting suspended sediment properties. *Water Air Soil Pollut*., **99**, 43-53.

Fatin-Rouge, N., Tóth, É., Perret, D., Backer, R.H., Merbach, A.E., and Bünzli J.-C. (2000). Lanthanide podates with programmed intermolecular interactions: Luminescence enhancement through association with cyclodextrins and unusually large relaxivity of the gadolinium self-aggregates. *J. Am. Chem. Soc.* **122**, 10810-10820.

Gustafsson, O. and Gschwend, P.M. (1997). Aquatic colloids: concepts, definitions, and current challenges. *Limnol. Oceanogr.,* **42**, 519-528.

Heissenberger, A., Leppard, G.G. and Herndl, G.J. (1996). Ultrastructure of marine snow. II. Microbiological considerations. *Mar. Ecol. Prog. Ser.,* **135**, 299-308.

Jackson, T.A., West, M.M. and Leppard, G.G. (1999). Accumulation of heavy metals by individually analyzed bacterial cells and associated nonliving material in polluted lake sediments. *Environ. Sci. Technol*., **33**, 3795-3801.

Jackson, T.A. and Leppard, G.G. (2002). Energy dispersive X-ray microanalysis and its applications in biogeochemical research. In: Violante, A., Huang, P. M., Bollag, J.-M. and Gianfreda, L. (eds): *Soil Mineral-Organic Matter Microorganism Interactions and Ecosystem Health.* Elsevier, Amsterdam; 219-260.

Jacobsen, C., Wirick, S., Flynn, G. and Zimba, C. (2000). Soft X-ray spectroscopy from image sequences with sub-100nm spatial resolution. *J. Microscopy*, **197**, 173-184.

Jullien R. (1992). The application of fractals to colloidal aggregation. *Croat. Chem. Acta*, 65, 215-235.

Kaegi R. (2003). In preparation.

Lawrence, J.R., Swerhone, G.D.W., Leppard, G.G., Araki, T., Zhang, X., West, M.M. and Hitchcock, A.P. (2003). Scanning transmission x-ray, laser scanning, and transmission electron microscopy mapping of the exopolymeric matrix of microbial biofilms. *Appl. Environ. Microbiol., **69,*** 5543-5554.

Leppard, G.G. (1992). Evaluation of electron microscope techniques for the description of aquatic colloids. In: Buffle, J. and van Leeuwen, H.P. (eds): *Environmental Particles, Vol. 1*. Lewis, Chelsea, MI; 231-289.

Leppard, G.G. (1995). The characterization of algal and microbial mucilages and their aggregates in aquatic ecosystems. *Sci. Total Environ.,* **165**, 103-131.

Leppard, G.G. (1997). Colloidal organic fibrils of acid polysaccharides in surface waters: electron-optical characteristics, activities and chemical estimates of abundance. *Colloids Surf. A*, **120**, 1-15.

Leppard, G.G., West, M.M., Flannigan, D.T., Carson, J. and Lott, J.N.A. (1997). A classification scheme for marine organic colloids in the Adriatic Sea: colloid speciation by transmission electron microscopy. *Can. J. Fish. Aquat. Sci.,* **54**, 2334-2349.

Leppard, G.G. and Buffle, J. (1998). Aquatic colloids and macromolecules: effects on analysis. In: Meyers, R.A. (ed): *The Encyclopedia of Environmental Analysis and Remediation, Vol. 1.* John Wiley & Sons, New York; 349-377.

Leppard, G.G., Flannigan, D.T., Mavrocordatos, D., Marvin, C.H., Bryant, D.W. and McCarry, B.E. (1998). Binding of polycyclic aromatic hydrocarbons by size classes of particulate in Hamilton Harbor water. *Environ. Sci. Technol*., **32**, 3633-3639.

Leppard, G.G. and Arsenault, A.L. (2003). Quantification of individual native biocolloids in natural waters: assessing indicators of aquatic events, by using transmission electron microscopy with a modified approach to image analysis. *Arch. Hydrobiol*., **156**, 565-573.

Leppard. G.G., Droppo, I.G., West, M.M. and Liss, S.N. (2003). Compartmentalization of metals within the diverse colloidal matrices comprising activated sludge microbial flocs. *J.Environ. Qual.* in press.

Liao, B.Q., Allen, D.G., Leppard, G.G., Droppo, I.G. and Liss, S.N. (2002). Interparticle interactions affecting the stability of sludge flocs. *J. Colloid Interface Sci*., **249**, 372-380.

Liao, B.Q., Bagley, D.M., Kraemer, H.E., Leppard, G.G. and Liss, S.N. (2003). Biofouling and its control in membrane separation bioreactors: a review. *Water Environ. Res.,* in press.

Lienemann, C.-P. (1997). Associations entre phases minérales de fer et de manganèse, polluants anthropogéniques et biota en milieu lacustre: mise en évidence par microscopie électronique. Ph.D. Dissertation, University of Lausanne.

Lienemann, C.-P., Taillefert, M., Perret, D. and Gaillard, J.-F. (1997a). Association of cobalt and manganese in aquatic systems: chemical and microscopic evidence. *Geochim. Cosmochim. Acta,* **61**, 1437-1446.

Lienemann, C.-P., Mavrocordatos, D. and Perret, D. (1997b). Enhanced visualization of polysaccharides from aqueous suspensions. *Mikrochim. Acta*, **126**, 123-129.

Lienemann, C.-P., Heissenberger, A., Leppard, G.G. and Perret, D. (1998). Optimal preparation of water samples for the examination of colloidal material by transmission electron microscopy. *Aquat. Microb. Ecol.,* **14**, 205-213.

Lienemann, C.-P., Monnerat, M., Dominik, J. and Perret, D. (1999). Identification of stoichiometric iron-phosphorus colloids produced in a eutrophic lake. *Aquat. Sci*., **61**, 133-149.

Lin, M.Y., Lindsay, H.M., Weitz , D.A., Ball, R.C., Klein, R., Meakin, P. (1989). Universality in colloid aggregation. *Nature*, 339, 360-362.

Liss, S.N., Droppo, I.G., Flannigan, D.T. and Leppard, G.G. (1996). Floc architecture in wastewater and natural riverine systems. *Environ. Sci. Technol.,* **30**, 680-686.

Mavrocordatos, D., Mondi-Couture, C., Atteia, O., Leppard, G.G. and Perret, D. (2000). Formation of a distinct class of Fe-Ca(-C_{org})-rich particles in a complex peat-karst system. *J. Hydrol.,* **237**, 234-247.

Mavrocordatos, D., Kaegi, R., Schmatloch, V. (2002). Fractal analysis of wood combustion aggregates by Contact mode Atomic Force Microscopy. *Atm. Env*., 36, 5653-5660.

Mondi, C., Leifer, K., Mavrocordatos, D. and Perret, D. (2002). Analytical electron microscopy as a tool for accessing colloid formation processes in natural waters. *J. Microsc*., **207**, 180-190.

Myneni, S.C.B., Brown, J.T., Martinez, G.A. and Meyer-Ilse, W. (1999). Imaging of humic substance macromolecular structures in water and soils. *Science*, **286**, 1335-1337.

Perret, D., Leppard, G.G., Muller, M., Belzile, N., DeVitre, R. and Buffle, J. (1991). Electron microscopy of aquatic colloids: non-perturbing preparation of specimens in the field. *Water Res*., **25**, 1333-1343.

Perret, D., Newman, M.E., Negre, J.-C., Chen, Y. and Buffle, J. (1994). Submicron particles in the Rhine River – I. Physico-chemical characterization. *Water Res.,* **28**, 91-106.

Perret, D., Gaillard, J.-F., Dominik, J. and Atteia, O. (2000). The diversity of natural hydrous iron oxides. *Environ. Sci. Technol*., **34**, 3540-3546.

Senesi, N. (1999). Aggregation patterns and macromolecular morphology of humic substances: a fractal approach. *Soil Sci.,* **164**, 841-856.

Taillefert, M., Lienemann, C.-P., Gaillard, J.-F. and Perret, D. (2000). Speciation, reactivity, and cycling of Fe and Pb in a meromictic lake. *Geochim. Cosmochim. Acta* **64**, 169-183.

Webb. S.M., Leppard, G.G. and Gaillard, J.-F. (2000). Zinc speciation in a contaminated aquatic environment: characterization of environmental particles by analytical electron microscopy. *Environ. Sci. Technol*. **34**, 1926-1933.

Weinbauer, M.G. and Peduzzi, P. (1994). Frequency, size and distribution of bacteriophages in different marine bacterial morphotypes. *Mar. Ecol. Prog. Ser.,* **108**, 11-20.

Wilkinson, K.J., Balnois, E., Leppard, G.G. and Buffle, J. (1999). Characteristic features of the major components of freshwater colloidal organic matter revealed by transmission electron and atomic force microscopy. *Colloids Surf. A*. **155**, 287-310.