# TRANSMISSION ELECTRON MICROSCOPY APPLIED TO WATER FRACTIONATION STUDIES - A NEW LOOK AT DOC by GARY G. LEPPARD

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## ABSTRACT

Research on the particle-mediated transport of "bound pollutants" tends to be conducted in the absence of a proper definition of particle and with a minimal consideration of colloidal phenomena. Recent developments in the use of transmission electron microscopy to monitor water fractionation schemes are providing a technology to correct this situation. This review summarizes the current status of the routine application of TEM in monitoring such schemes and in extending them into new areas of water research. It summarizes new findings on DOC relating to the extensive interpenetration of aggregated DOC "particles" with their aquatic milieu. Also, it relates colloidal phenomena to artifact generation in filter cascade schemes and in "particle-pollutant" interaction experiments. The usefulness of TEM in defining the ultrastructure of carbon-rich particulate materials and in elucidating colloid-particle interactions is demonstrated, as is the relevance of such measures to understanding pollutant dispersion.

### INTRODUCTION

A considerable research effort is being devoted to ascertaining the relationships between aquatic organic particles and toxic substances, with a focus on particle transport of "bound pollutants" (Kavanaugh and Leckie, 1980; Allan, 1984). This research is conducted in the context of an operational definition of particle (Wetzel, 1975) which is often inadequate (a particle is any material which will not pass a 0.45 µm filter). In the case of fine organic "particles" near the colloidal size range, this definition can mislead one's interpretation of experiments (Chase, 1979; Leppard, 1984b). Also, environmental scientists tend to conceptualize the transport status of an aquatic pollutant as being particle-associated or associated with the dissolved phase, without reference to colloidal phenomena. This is so despite published criticisms of this approach which go back one-half century (Ohle, 1935; Breger, 1970; Sharp, 1973) and a documentation of aquatic colloids which goes back even further (Wetzel, 1975). To ignore colloidal phenomena in the aquatic sciences now is to ignore a growing body of information showing that the organic colloid fraction of natural surface waters can be large (Wetzel, 1975; Leppard, 1984a) and that natural colloids are important in the dispersion of heavy metals (Florence and Batley, 1980) and organic pollutants (Means and Wijayaratne, 1982). Recent publications, described in the sections

below, suggest that colloidal carriers of pollutants could very well appear as soluble materials in one laboratory and as particulate material in another, depending on minor differences in sample handling and/or storage. Such an artifact problem can jeopardize attempts to relate particle-pollutant associations to sedimentation phenomena.

To address the problems of physical aftifact and misconception listed above and to provide a new look at DOC (the dissolved organic carbon fraction as conventionally-defined), this review summarizes:

- the status of the routine application of transmission electron microscopy (or TEM) in the monitoring of water fractionation studies;
   a synthesis of recent TEM findings on the nature of DCC.
- (2) a synthesis of recent TEM findings on the nature of DOC;
  (3) the rationale for considering the organic colloidal component of DOC as
- a fraction separate from the other organic components of DOC;
- (4) artifacts of sample preparation, experimentation and chemical interpretation encountered in applying TEM analyses to colloidal DOC;
- (5) some generalizations which relate biological, physico-chemical and colloidal phenomena to pollutant dispersion in surface waters.

## THE CURRENT STATUS OF TEM APPLICATIONS

Since the early days of the development of TEM and its associated technologies, biologists have been documenting the ultrastructure of biological materials taken from surface waters. By the 1960's, several studies of the colloidal extracellular materials of aquatic microbes were available in the literature (Leppard et al., 1977). However, the concepts and the technologies derived from such studies were transferred quite slowly into the fields of limnology and water pollution research (Leppard, 1984a). Recently, this situation has been changing with a major impetus being derived from studies revealing roles for aquatic colloids in the transport of heavy metals (Florence and Batley, 1980).

The use of TEM (in concert with optical microscopy and scanning electron microscopy) to monitor water fractions for cross-contamination artifacts is a relatively new area of enquiry (Leppard and Burnison, 1983). This monitoring application of TEM has been extended to investigations of the partitioning of environmentally-interesting organic materials within a given fractionation scheme. Some investigations on partitioning have led in turn to the isolation of specific colloids, such as lacustrine fibrils (Fig. 1), in high yield and purity (Burnison and Leppard, 1983, 1984). This latter development was put on a quantitative basis; it showed that fibrils alone can account for as much as 33% of the carbon in a conventional DOC fraction. Additionally, fibrils are an important constituent of the matrix structure of many biofilms (Geesey, 1982; Leppard, 1985), a fact which makes them a focus of interest for both limnologists and water treatment specialists.

Fibrils, whether they be suspended in a surface water or incorporated into a biofilm, will "bind" some aquatic pollutants, as a result of their chemical nature and polyanionic character (Geesey, 1982; Leppard and Burnison, 1983). Given that aggregated fibrils can exist in soluble, colloidal and particulate forms (Leppard, 1984a), then a control of fibril partitioning may be necessary in water fractionation schemes used to concentrate a fibril-associated pollutant for further analyses. Such a consideration is likely to hold true for some colloids other than fibrils (Leppard, 1983) and for soluble molecules which tend to form aggregates in concentrated solutions (Leppard <u>et al.</u>, 1985). ISOLATION OF COLLODAL FIBRILS FROM LAKE WATER



Figure 1 A generalized scheme (taken from Leppard and Burnison, 1983) for isolating fibrils from lake water, accompanied by some photomicrographic documentation by TEM of the nature of the separation achieved. Each level of the flow scheme of Figure IA can be monitored by TEM. Figure 1B shows a view of conventionally-defined DOC while Figure 1C shows a fibril-enriched fraction (the bar represents 0.45 µm).

The status of the routine use of TEM in 1985 can be summarized as follows:

- (1) cross-contamination of organic-rich fractions by fibrils, by colloidal granules and by tripartite membrane-like structures can be detected readily at better than 3nm resolution;
- (2) an improved design of filter cascade schemes, for producing more meaningful fractions, can be effected using results from TEM monitoring as guidelines;
- (3) it has become feasible, using a systematic trial-and-error process, to attempt the isolation of relatively pure fractions of specific kinds of organic colloids using TEM monitoring results as guidelines;
- (4) the principal artifact problems appear to be known, or at least recognizable, and they can now be investigated systematically (see later section);
- (5) the evolving technology can be extended to the study of biofilms (Leppard, 1985), DOC aggregation mechanisms (Leppard <u>et al.</u>, 1985) and colloidal organic costings on submerged surfaces (Leppard, 1984c);
- (6) TEM is showing the potential to be useful in relating the nature of water fractions and the chemical analyses of these fractions to an improved understanding of particle-mediated transport of "bound" pollutants - in short, it permits us to "see" relationships in both a physical and a conceptual sense.

### THE RATURE OF DOC AS SHOWN BY TEM

Among the components of conventionally-defined lacustrine DOC which can be visualized readily by TEM (those with at least one dimension greater than 0.0025 µm and at least two dimensions smaller than 0.45 µm), one finds certain morphological features which occur commonly (Burnison and Leppard, 1983). At the lower end of the size spectrum, most individual "particles" of DOC are extremely elongate and cannot be described readily in terms of an "effective radius" (see Fig. 1C). In this size range, the term "particle" is used in the general descriptive sense of the microscopist, and not in the sense of a sedimenting unit. At the upper end of the spectrum are many complex aggregated particles, some of which are readily sedimented. Such complex aggregates have features which could readily confound standard sedimentation analyses (Leppard, 1984b). These features are also found in the finest of the "true particles" (0.45 µg to 5.0 µm) and serve to blur further the distinction between particle and colloid, as set out by Vold and Vold (1964) who defined a colloid as having at least one dimension between 0.001 µm and 1.0 µm. The four most notable of these features grade into each other and are extremely difficult to quantify. They are described as follows:

- (1) considerable surface area per unit volume beyond that revealed by scanning electron microscopy, and accounted for by surface extensions and interpenetration of aggregate structure with the aquatic milieu;
- (2) great internal porosity, sometimes exaggerated to the extent that the pore volume exceeds the volume of visualizable structure;
- (3) a tenuous aspect, which can be so extreme as to blur the distinction between a given aggregated particle and an adjacent one (aggregated particles can interpenetrate each other so well as to give an impression that much of the carbon-rich material collected on a filter surface forms a continuum, as opposed to a layer of discrete units);
- (4) the frequent occurrence of fibrils which appear to serve as surface extensions and as adhesive structures which connect the various components of an aggregated particle.

When one views tens of thousands of TEM images of samples of colloidal DOC (and also of organic lacustrine particles in the finest size range), as has been done by several individuals at the National Water Research Institute of Canada, one can arrive at four strong impressions which are at variance with classical concepts of the nature of aquatic particles. First, the classical cutoff filter, with its 0.45 µm pore size is an inappropriate choice for defining the DOC fraction; it also fails to separate natural water samples into a cell-containing fraction and a cell-free fraction while failing to confine colloidal materials into one major fraction or the other. This choice of cutoff filter may have been useful in the past but its continued use will only serve as a source of confusion in future researches. Replacing it with a filter of 0.2 µm pore size would be advantageous to some researches now, but future research will demand the design of filter cascades which separate natural water samples into particulate, colloidal and dissolved components (see later section). Second, the impression of solidness, which one receives from the optical microscopy and scanning electron microscopy of fine organic particles, is replaced by an impression of delicacy and porousness at the greater resolution of TEM. Using the same comparisons, an impression of particle discreteness revealed by low resolution microscopy is replaced by the opposite impression when high resolution TEM is employed. This third impression is reinforced by the frequency of occasions where one cannot distinguish between a single aggregated particle and a collection of smaller aggregated particles found in such close proximity that they interpenetrate each other. Organic floc formation and organic particle growth may sometimes be no more than a physical entanglement of small tenuous aggregated particles and associated colloids, such as fibrils. The fourth strong impression is one of great extensions of surface area which are visualized only by the high resolution of TEM. Applications of the technology of surface chemistry would surely serve one well in studies of the colloidal component of DOC.

Considerable interest is now drawn to the study of organic coatings on aquatic particles, both from an ultrastructural standpoint (Leppard, 1984a) and a chemical one (Leppard, 1983). This interest is the result of certain

pioneering works on the behaviour of particles in marine surface waters (Neihof and Loeb, 1974; Hunter, 1980). This research showed clearly that particles in surface waters take on a coating of certain DOC materials and that such an organic coating is responsible for much of the chemical activity displayed by the particles. Currently, these findings are being extended to lake waters (Baccini et al., 1982). In lake gaters, sometimes the coating is colloidal and composed of DOC fibrils. Such lacustrine colloidal fibrils, of 0.004 µm to 0.018 µm diameter, can display selectivity in their attachment to natural surfaces; in one experimental lake study, these fibrils were shown to coat different kinds of surfaces to differing extents and tended to form incomplete coatings and/or porous coatings most of the time (Leppard, 1984c). The effect of these polysaccharide-rich fibril coatings on the coprecipitation of organic and inorganic particles could not be assessed quantitatively in this early study. 5

Among the finely dispersed materials of lake waters, the colloidal organic fibrils, of indeterminate length and narrow range of diameters, are currently the most amenable to TEM analyses, whether they be studied as a component of biofilms or as components of DOC fractions. Figure 2 diagrams their possible relationships to microbial activities, secreted polymers, floc formation, "pollutant binding", sedimentation phenomena, and the biological modulation of pollutant dispersion. This figure is speculative but the bases for constructing it are tenable (Leppard, 1984a) and it has considerable utility; its various components can be either proven or disproven using existing knowledge and technologies. Extending the scope of Figure 2 to marine and riverine ecosystems is feasible since some researchers claim to have detected fibrils as DOC components of coastal oceanic water and river water. As a rough guideline, fibril abundance is low where biological productivity is low (B.K. Burnison, personal communication).

The author is participating in some researches in progress which relate well to the theme of this paper. They can be summarized with respect to their goals as follows:

- (A) describing the physical effects of treatments, applied to DOC fractions and subfractions, which are routinely used to permit long-term storage of organic fractions;
- (B) describing the effects of concentrating humic substances, either by interaction with surfaces or by cascade filtration schemes, on colloid formation and/or aggregate formation, and the subsequent effects on metal scavenging activities;
- (C) describing catalytic activities of fibril preparations;
- (D) describing the dynamic status of aggregated particles at and near the colloidal size range.

All of the works above are progressing slowly. Some early findings, keyed to the researches described immediately above are worthy of notice. They are as follows:

- (A) freeze-drying can convert DOC fibrils and colloid-sized aggregates of them into densely-woven fibrous flakes and sheets, whose size can extend into the visible range;
- (B) pedogenic fulvic acids, in concentrated solutions, can leave true solution to form large colloidal aggregates, apparently by a dehydration mechanism;
- (C) fibrils can bind enzymes;
- (D) fibril-rich DOC preparations can be flocculated readily by interaction with multivalent cations, to form a floc which is readily dispersed by agitation (TEM observations suggest that one mechanism of floc formation by fibril-rich DOC is physical entanglement of small tenuous aggregated particles).



Figure 2 Fibrils, fibril components and fibril aggregates: their potential impacts on physico-chemical processes and microbial activities in natural waters. The bases for hypothesizing these relationships are found in Leppard (1984a).

## ORGANIC MATERIALS IN LAKE WATERS -ARE THERE TWO MAJOR FRACTIONS OR THERE?

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There is a sound scientific basis (Breger, 1970; Sharp, 1973; Leppard and Burnison, 1983) for fractionating surface waters in general and lake waters in particular, with respect to organic-rich materials, into three fractions (particulate, colloidal and dissolved) as opposed to the traditional two fractions of DOC and POC. Stated bluntly, there should be three major fractions in fractionation schemes because there are, in fact, three major classes of organic materials with regard to settling behaviour and solution/suspension phenomena. These can, of course, be subfractionated meaningfully along several obvious lines (e.g., cell-containing vs cell-free subfractions, or, readily sedimented colloids vs colloids responding to only the highest "conventional g forces").

The establishment of generally-accepted routine procedures to yield the three major fractions will not occur soon. With regard to settling behaviour, Chase (1979) demonstrated that small (5 µm to 500 µm) organicmineral aggregates did not settle in natural surface waters according to Stokes Law. Particles in the middle and upper portion of his "small particle" size range settled somewhat faster than expected, with lacustrine aggregates settling faster than marine ones. However, aggregates in the lowest portion of this size range (approaching colloidal dimensions) behaved differently, showing an order of magnitude increase in settling velocity over the Stokesian prediction. These results indicate that establishing a cutoff filter to distinguish betwen particles and colloids will not be so simple as applying the colloid definition of Vold and Vold (1964) directly to filter cascade schemes. Establishing a cutoff filter to realistically distinguish between colloidal and dissolved substances must also be researched much beyond a simplistic direct approach, taking into account many chemical and physical factors specific to a given water sample. This complication was probed recently by the TEM analyses of Burnison and Leppard (1983). They found that a small fraction of lacustrine DOC can be sedimented by high g forces despite the fact that the sedimentable portion of the DOC possessed some colloids having the same shape and dimensions as in the "truly dissolved" portion. Combination approaches involving both ultracentrifugation and filtration are showing promise in the author's laboratory but the work (to 1985) is still at a preliminary stage.

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#### PROBLEMS OF ARTIFACT

The imaging of DOC components, by TEM applied to ultrathin sections, covers almost the entire colloidal range  $(0.0025 \ \mu m to 0.45 \ \mu m as compared to$  $the range established by colloid chemists, 0.001 \ \mu m to 1.0 \ \mu m) and extends$ into the range of macromolecular aggregates. Most of what is visualized as"DOC particles" is probably so finely suspended that it would behave more asa solute than as a true particle. A small portion of it, however, is likelyto behave as a true particle in response to low g forces and/or dispersion bycurrent flow. With these qualifications in mind, there is clearly no way todesign a perfect DOC fractionation scheme; one can, at best, design onlyschemes which are approximately good enough to answer some specific researchneeds. Thus, this section will address itself not to the total spectrum ofartifacts, but rather to those artifacts which the scientist can minimize inproducing meaningful fractions. Physical artifacts leading to dehydrationinduced aggregation are of special concern in differentiating between"solute" and "particle" in natural waters (Leppard <u>et al.</u>, 1985). Sampling strategy and sample handling are important. For the isolation of a large quantity of a given colloid, such as a fibril fraction, it is advantageous to select a natural water enriched in that colloid. In the case of lacustrine fibrils, one's task can be simplified by sampling lake water shortly after collapse of a bloom of a fibril-producing alga. With regard to handling, there are some common-sense rules: one should start the TEM processing before the sample has time to "age"; one should avoid adding any chemical which would interfere with analyses at later stages; if a screening step is used to remove relatively coarse particles, then this step should be monitored to select a screen which does its task without contributing to any handling artifacts; if centrifugation is used at any place in the scheme as a concentrating step, then this step should be developed in conjunction with TEM monitoring to minimize both losses of colloid and artifacts of compaction.

The cytological chemical processing of colloid-rich organics for TEM is potentially a major source of artifact. The use of TEM for the purpose of monitoring a water fractionation scheme has, therefore, been understandably slow to develop. There is currently one methodology in the literature which has not been found wanting in its two-year existence (Burnison and Leppard, 1983) and it is employed routinely at the NWRI. The bases for this NWRI methodology are outlined briefly in Leppard <u>et al</u>. (1977); they are founded on many years of trial-and-error experimentation by numerous cytological and microbiological laboratories studying biological materials at the lower end of the particle size range and macromolecular aggregates in the colloidal size range. Because of the trial-and-error nature of TEM preparatory techniques, improvements in TEM monitoring and assessment are anticipated as this new field is expanded.

Artifacts of a fractionation process per se can be either chemical in nature or physical or a combination of both. In considering cascade filtration schemes (and derivative schemes), one must take into account certain constraints such as using only those "wash solutions" which have the least affect on the solubility of the materials being fractionated. If a denaturing solvent must be employed in a purification stage, it should be a solvent which has a minimal effect on the properties to be measured with the purified fraction. Physical artifacts in filter cascades can present subtle difficulties having chemical consequences. At the upper surface of fine filters, some natural organic solutes (such as fulvic acids) can become so concentrated that they aggregate spontaneously to form colloids (Leppard et al., 1985); thus there may be several components in a given filter cascade with a capacity to "create" colloids. This colloid "creation" activity could well bring about some respeciation of substances which had been associated with the dispersed organic prior to its aggregation. Such speculated relationships are worthy of much further research. Curiously, some apparently innocuous means of storage of water fractions (such as bulk freeze-drying, or storage as a concentrated solution/suspension) can result in aggregation processes which are reversible with difficulty or not at all (Leppard et al., 1985).

Once a fractionation scheme has been "fine tuned" (by guidelines established through TEM monitoring) to produce good yields of a given DOC component, the analyses of its chemical constitution and behaviour must be consistent with the special properties of the material. In essence, the chemical analysts receiving the fraction must be fully integrated into the overall study and its specific objectives to ensure that new artifacts are not introduced inadvertently and that interpretation is carried out in the context of the special properties. For example, analyses of manganese binding by colloids can yield confusing data if the chemist is not aware of

manganese oxidase activity in his colloid fraction. Analyses of flocculation phenomena in a colloidal suspension can yield artifactual data if the chemist inadvertently alters the physical nature of the suspension, an event which is a likelihood when an inappropriately-chosen storage treatment (or a denaturing pretreatment) causes changes in colloid hydration. In specific cases, such as fibril-metal interaction studies, one should keep in mind that organic colloids can be (and fibrils definitely are) extremely effective in poisoning electrodes.

The last-mentioned field of enquiry (fibril-metal interactions) is developing at a disappointingly slow pace. This is so despite a very strong rationale presented recently by many leading environmental researchers (and summarized well in Florence and Batley, 1980), a rationale which shows convincingly the urgency for better information on the role of aquatic colloids in heavy metal transport. In this author's opinion, the principal barrier to increasing the level of such research on aquatic colloids is fear of the associated artifact problems; this is manifested principally in a distrust of making a long-term commitment to open-ended research of a trialand-error nature. In particular, a slow pace in the analysis of fibril-metal interactions is often the result of trying to adjust to rather subtle physical artifacts which are revealed all too vividly by TEM monitoring. This problem is, potentially, a general problem to be addressed in "particle-pollutant interaction" experiments. It can be summarized as follows.

- (1) A given concentration of metal-free fibrils dissolved/suspended in water is used as a test system for the purpose of measuring the "binding" by fibrils of an experimentally applied heavy metal (such as iron or lead).
- (2) The test metal is applied to the test system.
- (3) The fibrils, with their associated metal, are then isolated from the test system.
- (4) The resultant fibril-metal association is analyzed for total metal, and the amount of bound metal per unit mass of fibrils is calculated.
- (5) It appears that interesting new data has been generated from a simple test system; then the results of TEM monitoring are assessed. These results reveal that the test system behaved in an unanticipatedly complex manner, one which precludes ready interpretation.
- (6) The TEM analysis does not reveal a situation so simple as one of fibrils having a uniformly increased electron density relative to controls (an index of metal binding). Instead, as a worst case scenario, one might see a meshwork of fibrils having a patchy distribution of electron density increases, with the meshwork enclosing electron dense granules which had not been present at the start of the assay test (and with some of these granules being attached to fibrils while others are merely encaged within the pore system of the meshwork).
- (7) Thus, one is left with two difficult problems to solve before one can proceed, namely, what fraction of the visualizable metal is artifactual and what fraction of fibril-associated metal can be properly considered as fibril-bound metal!
- (8) Finally, one is left with the insidious thought that the tests would have produced considerable "new data" (and such tests may be producing "new data" elsewhere) had one not taken the precaution of monitoring the test system by TEM.

### PARTICLES, COLLOIDS AND THE TRANSPORT OF POLLUTANTS IN ACUATIC ECOSYSTEMS

Particulate matter (including colloidal particles) plays major roles in the fate of pollutants in aquatic ecosystems. It can exert important effects upon the bioavailability, bioaccumulation, transport, recycling and burial of

many pollutant substances (Allan, 1984). Colloidal materials impinge on all of these phenomena (Florence and Batley, 1980; Leppard, 1983). For some time, it has been evident that the dispersion of organic contaminants can be mediated by colloidal phenomena, including organic colloids implicated in aggregation and sedimentation (Leshniowsky et al., 1970). Among the toxic heavy metals of concern to environmentalists today, at least four are dispersed in aquatic ecosystems to a great éxtent by organic colloids; these metals are cadmium, copper, lead and mercury (Leppard, 1984a). To understand more completely the aquatic dispersion processes, it is becoming imperative to separate colloidal impacts from those attributed to true solutes and to true particles. Water fractionation studies can and should be directed more towards this need for greater understanding, with TEM monitoring being developed as a useful accessory technology.

There is a tendency to separate organic particle phenomena from physiological phenomena in analyzing pollutant dispersion within aquatic ecosystems. This happens despite a growing literature on those biological processes which lead directly to the extracellular release of organics in response to toxic element insults (summarized in Leppard, 1984a). These processes include the secretion of organic macromolecules, colloids and floc-forming materials, all of which can contribute to particle formation as well as to changes in the surface activity, aggregation behaviour and sedimentation rate of particles. Figure 2 integrates some physiological phenomena with some mechanisms of particle formation and particle-mediated pollutant dispersal. It also diagrams some hypothetical central roles for colloidal organic fibrils which provide a context for future research endeavors. Such an integrated context for research can, of course, be recast to consider additional phenomena or to emphasize natural organics other than fibrils. Within such a context, one can probe more profoundly the physiological interplay between biota and pollutants in their aquatic milieu. This in turn will permit one to relate biological feedback activity, secretion, colloid formation, particle formation, availability regulation by biota, pollutant respeciation and particle-mediated dispersion of pollutants. Such a research approach is well described by the words of Farrington and Vandermeulen (1983) as one "in which new concepts and experimental procedures have emerged to tackle a problem that a reading of the literature of the past 20 yr indicates as a persistent, important void in knowledge of biogeochemical cycles".

#### KEY WORDS

Colloids, contaminant transport, fibrils, filtration.

## REFERENCES

- Allan, R.J. 1984. The role of particulate matter in the fate of contaminants in aquatic ecosystems: Part I. Transport and burial; Part II. Bioavailability, recycling and bioaccumulation. National Water Research Institute Contribution No. 84-18, Burlington, Ontario, Canada.
- Baccini, P., E. Grieder, R. Stierli and S. Goldberg 1982. The influence of natural organic matter on the adsorption properties of mineral particles in lake water. Schweiz. Z. Hydrol. 44, 99-116.
- Breger, I.A. 1970. What you don't know can hurt you: organic colloids and natural waters, p. 563-574. In D.W. Hood (ed.) Organic matter in natural waters. Institute of Marine Science, Occasional Publ. 1, College, Alaska.

Burnison, B.K. and G.G. Leppard 1983. Isolation of colloidal fibrils from lake water by physical separation techniques. Can. J. Fish. Aquat. Sci. 40, 373-381.

Burnison, B.K. and G.G. Leppard 1984. Bthanol fractionation of lacustrine

colloidal fibrils. Can. J. Fish. Aquat. Sci. 41, 385-388. Chase, R.R.P. 1979. Settling behavior of natural aquatic particulates. Limnol. Oceanogr. 24, 417-426.

Farrington, J.W. and J.H. Vandermeulen 1983. Retrospect and prospects, p. 357-360. In J.W. Farrington, J.H. Vandermeulen and D.G. Cook (eds.) Proceedings of the conference on pollution in the North Atlantic Ocean. Can. J. Fish. Aquat. Sci. 40(Suppl. 2), 1-362.

Florence, T.M. and G.E. Batley 1980. Chemical speciation in natural waters. CRC Crit. Rev. Anal. Chem. 9, 219-296.

Geesey, G.G. 1982. Microbial exopolymers: ecological and economic considerations. ASM News 48, 9-14.

- Hunter, K.A. 1980. Microelectrophoretic properties of natural surfaceactive organic matter in coastal seawater. Limnol. Oceanogr. 25, 807-822.
- Kavanaugh, M.C. and J.O. Leckie 1980. Particulates in water characterization, fate, effects, and removal, p. 1-401. American Chemical Society, Washington, D.C.

Leppard, G.G. 1983. Trace element speciation in surface waters and its ecological implications, p. 1-320. Plenum Press, New York.

Leppard, G.G. 1984a. Relationships between fibrils, colloids, chemical speciation, and the bioavailability of trace heavy metals in surface waters - A review. National Water Research Institute Contribution No. 84-45, Burlington, Ontario, Canada.

Leppard, G.G. 1984b. The ultrastructure of lacustrine sedimenting materials in the colloidal size range. Arch. Hydrobiol. 101, 521-530.

Leppard, G.G. 1984c. Organic coatings on suspended particles in lake water. Arch. Hydrobiol. 102, 265-269.

- Leppard, G.G. 1985. The fibrillar matrix component of biofilms Its structure can be explored as an index of biofilm function. (Submitted for publication).
- Leppard, G.G., J. Buffle and R. Baudat 1985. A description of the aggregation properties of aquatic pedogenic fulvic acids - Combining physico-chemical data and microscopical observations. Water Res. (Accepted for publication).
- Leppard, G.G. and B.K. Burnison 1983. Bioavailability, trace element associations with colloids and an emerging interest in colloidal organic fibrils, p. 105-122. In G.G. Leppard (ed.) Trace element speciation in surface waters and its ecological implications. Plenum Press, New York.
- Leppard, G.G., A. Massalski and D.R.S. Lean 1977. Electron-opaque microscopic fibrils in lakes: Their demonstration, their biological derivation and their potential significance in the redistribution of cations. Protoplasma 92, 289-309.
- Leshniowsky, W.O., P.R. Dugan, R.M. Pfister, J.I. Frea and C.I. Randles 1970. Aldrin: Removal from lake water by flocculent bacteria. Science 169, 993-995.

Means, J.C. and R. Wijayaratne 1982. Role of natural colloids in the transport of hydrophobic pollutants. Science 215, 968-970.

Neihof, R. and G. Loeb 1974. Dissolved organic matter in seawater and the electric charge of immersed surfaces. J. Mar. Res. 32, 5-12.

Ohle, W. 1935. Organische Kolloide in ihrer Wirkung auf den Stoffhaushalt der Gewasser. Naturwissenschaften 23, 480-484.

Sharp, J.H. 1973. Size classes of organic carbon in seawater. Limnol. Oceanogr. 18, 441-447.

Vold, M.J. and R.D. Vold 1964. Colloid chemistry, the science of large molecules, small particles, and surfaces, p. 1-118. Reinhold Publ. Corp., New York.

Wetzel, R.G. 1975. Limnology, p. 1-743. W.B. Saunders Co., Philadelphia.