# Appropriateness of *Aufwuchs* as a Monitor of Bioaccumulation

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#### ABSTRACT

Aufwuchs, procedurally defined as material accumulating on submerged surfaces, is being used increasingly to monitor trace element bioaccumulation in aquatic biota. Procedurally-defined aufwuchs is a complex mixture of biotic and abiotic components. Both biotic and abiotic components can be avid concentrators of trace elements. Consequently, bioaccumulation data generated from poorly-characterized, procedurally-defined aufwuchs may not accurately reflect accumulation by biota. Further, total concentrations of trace elements in procedurally-defined aufwuchs may not be indicative of the amount of contaminant available for trophic transfer. Methods of minimizing abiotic component contribution to trace element accumulation and means of assessing the bioavailability of associated trace elements are discussed in this review.

### DESCRIPTION OF METHODS

#### Overview

Sladeckova (1962) noted that *aufwuchs* have been used in taxonomic surveys, ecological investigations, productivity measurement and applied

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hydrobiology. This review will examine a rapidly growing use of *aufwuchs* in the area of applied hydrobiology. Such studies use *aufwuchs* community structure or processes, and *aufwuchs* composition to monitor response to contamination. The coverage and focus of the literature review reflect our intent to define important concepts, not compile the present body of literature.

# **Terminology**

The *aufwuchs* is that assemblage of organisms growing attached to, or clinging upon, free surfaces of submerged objects such as stems, roots and leaves of living plants, rock, wood and animals (Sladeckova, 1962; Odum, 1971). Weitzel (1979) distinguishes between the etymologically more comprehensive term, *aufwuchs* ('those organisms that are firmly attached to a substrate and including those free-living forms within the mat but not penetrating into it'), and the more restricted term, periphyton ('all aquatic organisms (microflora) growing on submerged substrates'); however, these terms have become synonymous in recent use.

During many studies of biomass or contaminant accumulation, the aufwuchs are procedurally-defined as material accumulating on submerged surfaces (Newman et al., 1985). Under this procedural definition, tychoplankton (planktonic forms normally characteristic of the open waters), dead organisms, previously suspended inorganic and organic material, and other surface-derived abiotic materials may be included in the sample (Weitzel, 1979; Newman, 1983; 1985). To avoid confusion, the terms aufwuchs (Weitzel, 1979) and procedurally-defined aufwuchs (Newman, 1985) will be used in this review to distinguish between the biological community and all materials covering submerged surfaces, respectively.

# Water quality assessment

The most frequent use of aufwuchs in assessing the effects of altered water quality is identification of community or species shifts (Evenson et al., 1981). Some researchers discuss alterations in broad taxonomic groupings, while others focus on particular, sensitive species. Chadwick & Canton (1983) noted no shifts in broad taxonomic groups during a survey of aufwuchs below a coal strip-mine. In contrast, Patrick (1978) demonstrated a clear shift from diatoms to green or blue-green algae during controlled experimental exposure of aufwuchs to vanadium. Patrick suggested that the associated shift from high quality food items such as diatoms to lower quality food such as green algae could significantly affect the grazing food web during contaminant exposure. Other field studies, such as those conducted in an oligotrophic lake receiving metals from mine drainage

(Roch et al., 1985; Austin & Deniseger, 1985; Austin et al., 1985) also have identified shifts in species composition, with loss of metal-sensitive species.

Controlled exposures to organic contaminants (Holm et al., 1983; Scott, 1984; Scott et al., 1984; Goldsborough & Robinson, 1986), trace elements (Sigmon et al., 1977; Patrick, 1978; Kaufman, 1982) and complex effluents (Gerhart et al., 1977) suggest that aufwuchs biomass (presumably procedurally-defined aufwuchs ash free weight), species composition, successional processes and diversity can be effective indicators of contaminant impact during properly conducted field surveys. Further, community characteristics, such as stress resistance and resilience (Kaufman, 1982), and community processes, such as net productivity (Tilley & Haushild, 1975; Hamala & Kollig, 1985), autotrophic and heterotrophic production (LeLand & Carter, 1985), photosynthesis (Blanck, 1985), nitrogen fixation (LeLand & Carter, 1985), and nutrient cycling (Malanchuk & Kollig, 1985) provide additional information with which to assess the effects of contaminants on aquatic biota. Although methods for quantifying growth rates on submerged surfaces have been formulated for microflora (Caldwell et al., 1981), this important characteristic has not been evaluated directly in studies of contaminant effect on aufwuchs. The extensive literature associated with field sampling of aufwuchs (Weitzel, 1979, 1983), and field and laboratory microcosms (Gerhart et al., 1977; Bothwell, 1983; Hamala & Kollig, 1985) may be linked with available measures of aufwuchs structure and function to provide a valuable means for contaminant monitoring.

The high concentration factors (concentration in the organism/concentration in the source) for trace elements noted for algae (Trollope & Evans, 1976) and bacteria (Johnson *et al.*, 1981) suggest a further valuable role for *aufwuchs* in monitoring these contaminants (Johnson *et al.*, 1978; Friant & Koerner, 1981; Ramelow *et al.*, 1987). Available information gleaned from *aufwuchs* monitoring can be enhanced by measuring bioaccumulation as will be discussed within this review.

# Bioaccumulation of trace elements

Procedurally-defined aufwuchs are frequently selected as the monitor of choice for contaminant bioaccumulation in aquatic environs. Several factors account for this selection. Firstly, natural or artificial substrates are easily sampled; thus the difficulties associated with obtaining sufficient biological material during all seasons can be circumvented. When artificial substrates are used, they are inexpensive, and easily fabricated and maintained. Secondly, algae and other microflora comprising the aufwuchs community exhibit high concentration factors for many trace elements

(Trollope & Evans, 1976; Patrick & Loutit, 1977). Thirdly, this microfloral community plays a potentially important role in the trophic transfer of contaminants. Fourthly, bioaccumulation data for aufwuchs can be readily combined with results from community composition or processes studies. Fifthly, there exists a plethora of investigations of the aufwuchs in freshwater, estuarine and marine systems including major reviews of methods (Weitzel, 1979, 1983). Sixthly, as a community is being monitored, the ecological realism associated with any supplemental laboratory studies is superior to analogous, single species assays (Blanck, 1985). Seventhly, during studies employing microcosms or enclosures, the establishment of a viable aufwuchs community is easy and, often, necessary to support other significant species that graze on aufwuchs. These factors have led to a final advantage: the existence of numerous studies employing aufwuchs as bioaccumulators which can be drawn upon for comparison. By consequence, aufwuchs have been used in marine, estuarine and freshwater (lotic and lentic) systems to provide a time-integrated estimate of biologicallyavailable trace elements to a microfloral community of trophic significance.

The *aufwuchs* ranks favorably relative to the criteria for the ideal biological indicator organism as summarized by Phillips (1977). Two criteria ('A simple correlation should exist between the metal content of the organism and the average metal concentration in the surrounding water' and 'That all organisms in a survey exhibit the same correlation between their metal contents and those in the surrounding water, at all locations studied, under all conditions') are difficult to verify for any candidate organism and such factors as pertinent to the use of *aufwuchs* in monitoring trace elements will be discussed herein.

## PROCEDURALLY-DEFINED AUFWUCHS

# Field surveys

Hutchinson et al. (1975) employed procedurally-defined aufwuchs to monitor bioaccumulation of copper, nickel and zinc released from mining activities in the Sudbury region (Canada). When several species of rooted macrophytes, zooplankton, fish, crayfish, clams and procedurally-defined aufwuchs were sampled, trace element concentrations in procedurally-defined aufwuchs were among the highest measured. For example, highest concentrations of nickel were associated with the procedurally-defined aufwuchs. Although some macrophytes and crayfish did have apparent concentration factors for copper similar to or higher than those for procedurally-defined aufwuchs, it was not obvious whether the potentially

significant contribution of epiphytic or epizooic aufwuchs to macrophyte and crayfish copper concentrations was considered (Patrick & Loutit, 1977; Johnson et al., 1981; Smock, 1983). Johnson et al. (1978) used plexiglass substrates to examine the value of procedurally-defined aufwuchs to reflect the intensity of trace element contamination in two lakes receiving wastes from electroplating facilities. They suggested that, when differences in contamination were significant, procedurally-defined aufwuchs were useful in monitoring bioavailability. Bioaccumulation data were linked to a general consideration of contamination tolerant and intolerant algal species. The materials analyzed were considered primarily biotic during both studies, and assessment of abiotic factors that could have influenced the accumulation of trace elements in these materials was generally lacking. Cushing et al. (1981) examined radionuclide (60 Cobalt and 65 Zinc) decrease in biota below plutonium production reactors after operation was discontinued. Procedurally-defined aufwuchs activities were generally higher than those of other components (seston, caddisfly larvae, suckers and squawfish). The loss rate of radionuclide activities from these procedurallydefined aufwuchs was thought to result from the following factors: (1) physical decay of the radionuclide; (2) biological turnover (elimination); and (3) decreasing availability in the water. No discussion of other removal mechanisms including abiotic mechanisms was presented.

In contrast to the above studies in which the contaminants were considered to be associated primarily with the biota, Evans & Giesy (1978) suggested from a study of bioaccumulation of cadmium, chromium, copper, iron, manganese, nickel, lead and zinc from a coal ash basin effluent that most of the trace elements measured in the procedurally-defined aufwuchs were associated with impacted particles enmeshed in the aufwuchs. Friant & Koerner (1981) and Ramelow et al. (1987) acknowledged a partial contribution of abiotic material to the procedurally-defined aufwuchs but concluded that biological mechanisms dominated accumulation of trace elements on glass slides. Ramelow et al. (1987) concluded that the contribution of suspended sediments to elemental concentrations in procedurally-defined aufwuchs was minimal, because sediment concentrations were significantly lower than those in the procedurally-defined aufwuchs.

#### Microcosm studies

Microcosm and laboratory studies have frequently employed procedurally-defined *aufwuchs* as a major biotic component during studies of the fate and effect of organic (Leversee *et al.*, 1980; Bruno *et al.*, 1982; Bartell *et al.*, 1984) and inorganic (Patrick, 1978; Gachter & Geiger, 1979; Bowling *et al.*, 1980;

Giesy et al., 1981) contaminants. Most of these workers assume during interpretation and modeling efforts that the procedurally-defined aufwuchs is primarily biological material; however, it was likely that, in some of these studies, inorganic components, such as hydrous iron oxides, could have played major roles in accumulation of contaminants (H. Kania, pers. comm.).

Some microcosm studies have focused on important aspects of contaminant accumulation in procedurally-defined *aufwuchs* other than for use as a monitor of readily-available contaminants. For example, Vymazal (1984) demonstrated the effectiveness of procedurally-defined *aufwuchs* in removing trace elements from an effluent. Phelps & Mihursky (1986) used laboratory manipulations of procedurally-defined *aufwuchs* to determine effective concentrations of copper for inhibition of oyster spat set and metamorphosis.

# Potential for misinterpretation of bioaccumulation data

In a survey of two New Jersey reservoirs, procedurally-defined aufwuchs had the highest concentrations of lead of all biotic components surveyed (Newman & McIntosh, 1982), including filamentous algae. However, concentration factors calculated for procedurally-defined aufwuchs in each reservoir were dissimilar and no significant correlation was found between changes in *aufwuchs*-associated lead concentrations and those of several surface grazing gastropods. Further analysis of these materials revealed that 8 to 11% (w/w) of the procedurally-defined aufwuchs was iron plus manganese (Newman et al., 1983). Implications drawn from correlation analyses (i.e. more lead was associated with an iron and manganese-rich matrix surrounding the microflora than the microflora itself), were confirmed using energy dispersive X-ray fluorescent spectrometry/scanning electron microscopy. The authors suggested that hydrous iron and manganese oxides, avid concentrators of trace elements in soils and sediments (Jenne, 1968), were responsible for most of the accumulation of lead by these 'biological materials'. The generality of these findings was fostered by similar results in a South Carolina system receiving coal ash basin effluent (Newman et al., 1985). The potential for generating invalid bioaccumulation data using procedurally-defined aufwuchs is evident from the results of these studies.

## Role of biotic and abiotic mechanisms

Neal et al. (1967) used procedurally-defined aufwuchs to estimate the level of radionuclide contamination within a small Tennessee lake, White Oak Lake.

The interpretation of these data was based primarily on biological mechanisms. In 1982, Cerling and Turner examined radionuclide accumulation in iron and manganese coatings (procedurally-defined aufwuchs) in the stream draining into this lake. Although the materials were collected from submerged surfaces within the same watershed studied by Neal et al. (1967), Cerling and Turner interpreted the radionuclide accumulation results primarily in geochemical terms. Indeed, a cursory review of the biogeochemical literature reveals a significant number of studies employing submerged surface coatings (procedurally-defined aufwuchs as described herein) for geochemical exploration (Carpenter & Hayes, 1980; Robinson, 1981; Filipek et al., 1981). Each geochemical study downplays the role of biotic mechanisms in contaminant accumulation in procedurally-defined aufwuchs just as the biological studies tend to downplay the significance of abjotic mechanisms. In reality, procedurally-defined aufwuchs is a complex and variable mixture of biotic and abiotic components; therefore, the accumulation of contaminants by this material results from a suite of interrelated biogeochemical mechanisms.

A variety of physical and chemical mechanisms have been identified for the gain and loss of contaminants by procedurally-defined *aufwuchs* (Fig. 1). Obviously, particle impaction (Evans & Giesy, 1978), coating abrasion

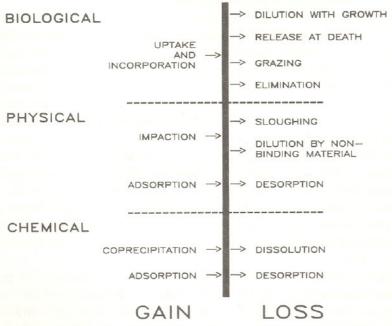


Fig. 1. Biological, physical and chemical mechanisms contributing to the accumulation of trace elements in procedurally-defined *aufwuchs*.

(Cerling & Turner, 1982) and sloughing (Cerling & Turner, 1982) can significantly influence accumulation of mass (biomass?) or contaminants by procedurally-defined *aufwuchs*. Water flow, suspended solids load, composition of the suspended solids, nature and orientation of the substrate are particularly important variables to consider and define during experimental design and data analysis, especially in studies of lotic systems.

In lentic systems, ecton can form an overlaying flocculent layer during periods of calm weather and influence lead concentrations in procedurallydefined aufwuchs (Everard & Denny, 1985). In these studies, the ecton and procedurally-defined aufwuchs formed a continuum, with the distinction between the two phases being defined solely by the degree of attachment to the substrate. Materials, including lead-laden particulates, were exchanged readily between the two phases. Settling of such materials should be minimized or quantified in studies in lentic systems or depositional environments. The material added to the mass of the procedurally-defined aufwuchs may elevate or lower the apparent concentration of the contaminant, depending on the nature of the material. If the contribution of seston or ecton to the procedurally-defined aufwuchs is controlled, caution must be exercised during discussions of metal bioavailability. The amount of contaminant in materials on the natural submerged surfaces and available for grazing by stream biota may not be the same as that for the substrates with controlled seston or ecton deposition.

Coprecipitation of trace elements with hydrous oxides (Inoue & Munemori, 1979; Kinniburgh & Jackson, 1981) provides another chemical mechanism for elemental accumulation by procedurally-defined *aufwuchs*. Selectivity of coprecipitation (and adsorption) under various conditions for some heavy metals is given in Table VII of Kinniburgh & Jackson (1981).

Physical and chemical adsorption may provide a significant means of contaminant accumulation in procedurally-defined *aufwuchs*. Adsorption may be to cell surfaces (Fujita & Hashizuma, 1975), clays (Jenne, 1968; Sakata, 1987), hydrous manganese and iron oxides (Jenne, 1968; Inoue & Munemori, 1979; Kinniburgh & Jackson, 1981; Thanabalasingam & Pickering, 1985; Pierce & Moore, 1982; Sakata, 1987; Zachara *et al.*, 1987), associated organic matter (Filipek *et al.*, 1981), or carbonates (Jenne, 1968). Adsorption to clays and oxides may be complicated by associated organic compounds that modify these surfaces (Davis & Leckie, 1978; Davis, 1982; Laxen, 1985).

Adsorption and desorption of trace elements are influenced by physiochemical characteristics of the water body in question and the surface microlayer. The *aufwuchs* itself can modify the pH, eH, oxygen and carbon dioxide concentration in the surface microlayer via photosynthetic and respiratory activities and, consequently, modify the abiotic character of the

procedurally-defined *aufwuchs* by enhancing precipitation/dissolution of hydrous iron and manganese oxides or adsorption/desorption of contaminants. Further, hydrous iron oxides on submerged surface coatings can significantly influence species number, biomass and gross productivity of *aufwuchs* (Sode, 1983).

Dissolved organic compounds that form complexes or chelates with trace elements (Davis, 1982) and inorganic ions which influence metal speciation (Inoue & Munemori, 1979; Pierce & Moore, 1982; Thanabalasingam & Pickering, 1985; Zachara et al., 1987) can also modify adsorption of trace elements to surface coating components. Organic compounds adsorbed to surfaces may enhance trace element adsorption by stabilizing the adjacent surface sites and by providing new sites for adsorption (functional groups) (Davis & Leckie, 1978). There are suggestions that complexation of elements by adsorbed organics is stronger than that by dissolved organic compounds (Laxen, 1985); therefore, elevated dissolved organic matter in aquatic systems could enhance, more than hinder, elemental association with surface coatings. In contrast, ligands such as the chloride ions that do not readily adsorb onto surfaces will decrease the amount of trace element adsorbed to a solid surface (Davis & Leckie, 1978).

Adsorption of metals and metalloids can be described with Langmuir isotherms for hydrous metal oxides (Pierce & Moore, 1982) or clays (Kinniburgh & Jackson, 1981). If bonds involved in adsorption are quite strong (chemical adsorption), desorption may be minimal. Trace elements experiencing physical adsorption via weak bonds such as those involving van der Waals forces may readily undergo desorption. Assuming constancy of nature, adsorption of trace elements to procedurally-defined *aufwuchs* could be described by Langmuir (or Freundlich) isotherm equations. The amount of contaminant adsorbed would be directly proportional to the concentration in the medium (assuming equilibrium conditions). Whether the composition of the materials renders invalid the assumptions on which these equations are based remains unresolved in most studies. Heterogeneity of available adsorption sites, little reversibility of adsorption and constancy in the number of binding sites may also invalidate the use of these equations and associated conclusions.

Adsorption kinetics are often characterized by an initial, rapid adsorption and then a slower adsorption, limited by diffusion into the material (Rose & Cushing, 1970; Kinniburgh & Jackson, 1981). This occurrence suggests that thickness of the procedurally-defined *aufwuchs* can significantly influence adsorption of trace elements. Bioaccumulation data associated with short-term exposures in the field (short duration releases, for example) or short-term spiking in laboratory experiments may be more accurately presented on an areal than a gravimetric basis. The occurrence of this effect of

procedurally-defined aufwuchs thickness also implies that experimental design can significantly influence the results. For example, in study sites characterized by rapid accumulation of procedurally-defined aufwuchs or pulsed releases of contaminants, frequent collection and replacement of substrates that have accumulated relatively thin surface coatings in short periods of time may be preferable to continuous subsampling of a series of substrates that are placed into the water at the same time. For example, Neal et al. (1967) noted a hyperbolic relationship between radionuclide activity-density and biomass in procedurally-defined aufwuchs. Assumptions of constant bioconcentration factors or description with Langmuir isotherms would predict a linear relationship. Although this effect could have been produced by dilution of the activity per unit mass of the materials during rapid growth, diffusion-limited adsorption probably contributed to this deviation (Rose & Cushing, 1970). Such diffusion-limited adsorption can detract from the value of procedurally-defined aufwuchs as providers of a time-integrated estimate of contaminants in a study system. The effects of coating thickness on adsorption kinetics could be exacerbated if substrates are allowed to develop increasingly thicker coatings with time. If microbially-mediated changes in microlayer physiochemistry are brief (i.e. active periods of photosynthesis), then the time available for effective diffusion and avid adsorption deep within the matrix may be limited, regardless of the general constancy of contaminant concentrations outside of the surface microlaver.

Biological processes are often the primary mechanisms implicated in contaminant accumulation in procedurally-defined aufwuchs. The bioconcentration factor is assumed to be relatively constant, despite the myriad of species present and potentially rapid shifts in dominants in this community. Further, wide shifts in physico-chemical conditions in the surface microlayer associated with microfloral activity could provide a significant change in the relative roles of abiotic and biotic components in contaminant accumulation. Fujita & Hashizume (1975) described the sequence of cell surface adsorption, uptake and incorporation of mercury by the freshwater diatom, Synedra ulna. At high concentrations, contaminants can modify membrane processes such as uptake or elimination (Mayfield & Munawar, 1983). The concentration of a contaminant can decrease during periods of active growth (Neal et al., 1967; Rose & Cushing, 1970; Fujita & Hashizume, 1975). If the biomass to which the concentration is standardized increases significantly, growth will produce an apparent 'dilution' of the contaminant. Trace elements can also be lost by active elimination, grazing or release at death.

The validity of monitoring bioavailability using procedurally-defined aufwuchs remains uncertain if the mechanisms involving abiotic compo-

nents are not carefully controlled, assessed or defined. In several studies (Newman *et al.*, 1983, 1985), the abiotic components played a dominant role in contaminant accumulation. Therefore, many of the cursory interpretations present in the literature regarding bioaccumulation in procedurally-defined *aufwuchs* could be as misleading as the notion of 'bioaccumulation' by sediments or suspended solids.

#### BIOAVAILABILITY

A dominant theme in studies of trace element bioaccumulation by procedurally-defined *aufwuchs* is the assumed epiphenomenal trophic transfer of associated contaminants. However, the information reviewed to this point suggests that the assumption of readily-transferable contaminant to grazers may be invalid. Procedurally-defined *aufwuchs* cannot be considered to be biological tissue; indeed, procedurally-defined *aufwuchs* is a complex mixture of abiotic and biotic components. As such, the question of bioavailability to grazers becomes similar to that regarding bioavailability of sediment-bound contaminants. Newman & McIntosh (1983) suggested that lead in procedurally-defined *aufwuchs* was much less available for accumulation by a grazing snail than lead in plant tissue (lettuce leaf).

Assessment of the bioavailability of contaminants in procedurally-defined *aufwuchs* could be made in much the same manner as for sediment-bound contaminants. Particularly significant in this comparison is the role of sediment-associated iron oxides in controlling bioavailability as hydrous oxides of this metal are major concentrators of elements in procedurally-defined *aufwuchs* (Newman *et al.*, 1983, 1985). Studies of sediment-bound metals (Jenne & Luoma, 1977; Luoma & Jenne, 1977; Luoma & Bryan, 1978; Cooke *et al.*, 1979; Tessier *et al.*, 1984; Lewis & McIntosh, 1986) and metalloids (Langston, 1980) suggest that the iron and perhaps manganese oxide-associated elements are among the least bioavailable forms.

Some forms of organically-complexed cadmium were also found to be relatively unavailable to the polychaete, *Nereis diversicolor* as well (Jenne & Luoma, 1977). However, elements associated with clay (bentonite) (Phelps, 1979) and organic debris (Jenne & Luoma, 1977; Luoma & Jenne, 1977) were relatively available for accumulation by this polychaete and the bivalve, *Macoma balthica*. Several studies (Luoma & Bryan, 1978; Langston, 1980; Lewis & McIntosh, 1986) suggest that the ratio of metal to iron in a 1n HCl or 25% (v/v) acetic acid extract of sediments accurately predicts the relative bioavailability of trace elements in sediments. The higher this ratio becomes, the more available the trace element for bioaccumulation. Luoma & Bryan (1978) suggest that, with materials with low metal to iron ratios, the regression between concentration in the biota and ratio in the sediment is

best performed after log transformation of the data. This transformation avoids the occasional, negative Y-intercept after regression analysis.

The total elemental concentration in procedurally-defined aufwuchs is likely not the most effective measure of bioavailable contaminant. Using the ratio of metal to iron in an extract as described above could provide a more accurate assessment of bioavailability in systems where iron is a dominant actor in trace element accumulation in procedurally-defined aufwuchs. This approach remains untested at present. A variety of extraction methods are available for experimentation (Tessier et al., 1980, 1984); however, it is important to understand the limitations of these procedures during their use and during interpretation of associated data (Rendell et al., 1980; Tipping et al., 1985; Rapin et al., 1986; Tessier & Campbell, 1988; Bauer & Khebojan, 1988). The use of these extraction methods, perhaps in combination with mechanical or detergent treatments, could provide information sufficient for empirically assessing the bioavailability of contaminants from this complex, vet extremely important, component of aquatic systems. We are not aware of any attempt to implement this approach for trace elements in procedurally-defined aufwuchs.

#### SUMMARY

 Aufwuchs species and community characteristics provide a valuable means for contaminant impact monitoring.

(2) Procedurally-defined aufwuchs is a complex mixture of abiotic and biotic components. Both abiotic and biotic components can be avid concentrators of inorganic contaminants.

(3) Bioaccumulation data generated for poorly-characterized, procedurally-defined *aufwuchs* may not reflect accumulation by biota. Data from such studies have questionable value.

(4) Abiotic factors influencing inorganic contaminant accumulation must be minimized or clearly assessed in the experimental design, and their relative roles incorporated into the discussion of the results.

- (5) The thickness of the procedurally-defined aufwuchs can modify concentration factors for trace elements. This potentially complicating factor can be minimized by exposing substrates for relatively short periods of time or expressing the contaminant levels on an areal basis as well as a gravimetric basis. This problem could be most prominent when contaminant concentrations or physico-chemical conditions change at a rate that allows diffusion-limited adsorption kinetics to become significant.
- (6) Total concentrations in procedurally-defined aufwuchs are inadequate for judging the potential trophic transfer of trace elements.

Alternate measures of bioavailable contaminant such as those developed for sediment-bound contaminants have not been adequately assessed or utilized for procedurally-defined aufwuchs.

(7) Critical areas requiring further research include assessments of the relative importance of biotic and abiotic components in determining trace element concentrations in procedurally-defined aufwuchs, definition of methods to minimize the role of abiotic factors in accumulation of trace elements in procedurally-defined aufwuchs, examination of the bioavailability of trace elements associated with procedurally-defined aufwuchs, and methods development for predicting bioavailability of associated trace elements.

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