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GEOCHEMICAL FACTORS COMPLICATING THE USE OF *AUFWUCHS* TO MONITOR BIOACCUMULATION OF ARSENIC, CADMIUM, CHROMIUM, COPPER AND ZINC

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Abstract—Material accumulating on submerged glass slides was sampled from five sites associated with coal ash settling basins and a control site. Correlation analyses demonstrated a strong, positive correlation between the five elements, arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu) and zinc (Zn) and concentrations of associated iron or manganese. Negative, weak or nonsignificant correlations were noted between the concentrations of these five elements and microfloral cell densities or per cent ash free weight of the material. Scanning electron microscopy and *X*-ray analyses indicated that the majority of the material was abiotic and the elemental levels associated with the abiotic components were generally higher than those of the biotic components. Hydrous iron and manganese oxides likely play dominant roles in determining the trace element concentrations in these procedurally-defined *aufwuchs*. These findings indicate the potential for misinterpretation of biomonitoring data employing procedurally-defined *aufwuchs*.

Key words—*aufwuchs*, iron oxides, manganese oxides, chromium, arsenic, cadmium, copper, zinc

INTRODUCTION

Aufwuchs, procedurally defined as material accumulating on submerged substrates, are being used increasingly in trace element biomonitoring efforts (Evans and Giesy, 1978; Johnson *et al.*, 1978; Friant and Koerner, 1981). Two major factors contribute to the potential value of this approach. First, algae display very high concentration factors for many trace elements (Kenney *et al.*, 1976; Denney and Welsh, 1979). Second, substrates are easily fabricated, maintained and sampled thus circumventing many of the difficulties involved in procuring sufficient biological material during all seasons. Together, these two factors have given rise to increased biomonitoring with procedurally-defined *aufwuchs* and an extensive data base for comparisons. However, Newman *et al.* (1983) suggested that iron and manganese oxides accumulating on substrate surfaces may contribute more to the metal concentrations of the procedurally-defined *aufwuchs* than the microfloral community. As Odum (1971) defines *aufwuchs* as "organisms attached or clinging to stems or leaves of rooted plants or other surfaces projecting above the bottom", the results from studies employing procedurally-defined *aufwuchs* (material accumulating on slides) may not accurately assess the amount of a specific element in the *aufwuchs* (biota). The increasing use of procedurally-defined *aufwuchs* and the ambiguity regarding the relative importance of nonbiological versus biological concentration mechanisms prompted an examination by the authors of material accumulating on slides in a coal ash basin and its receiving waters.

MATERIALS AND METHODS

Sampling sites

Six sampling sites were selected at the Savannah River Plant (South Carolina, U.S.A.). Five (Sites A–E) were associated with the 400D Area coal power plant ash basins while an additional site (F) was established in the relatively uncontaminated Par Pond. The sites associated with the ash basins are shown in Fig. 1. Sluiced coal ash from the power plant is pumped into a 14.6 ha settling basin (calculated retention time: 39 days). The waters of this basin then empty into a 6.1 ha basin (calculated retention time: 22 days). The effluent of this second basin passes though a flume and into a large swampy area (approx. 2.4 ha). The water then flows down a narrow channel into Beaver Dam Creek. The effluent from the basins is diluted approx. 20-fold by the Beaver Dam Creek waters. The water then enters the Savannah River Swamp and, eventually, into the Savannah River. Site A is located in the 6.1 ha settling basin. Site B is located at the outfall of this basin while site C receives the effluent from the large swampy area (approx. 250 m below the outfall). Respectively, sites D and E are approx. 300 m and 1 km below the confluence of the effluent stream and Beaver Dam Creek.

Aufwuchs

Floating racks were designed to hold large numbers of glass slides in a vertical position fifteen centimeters below the waters surface (Fig. 2). The racks were inexpensive, easily made and sufficiently durable to withstand the rigors associated with flowing systems. Two plastic slide boxes (100 slide capacity each) with panels removed as illustrated
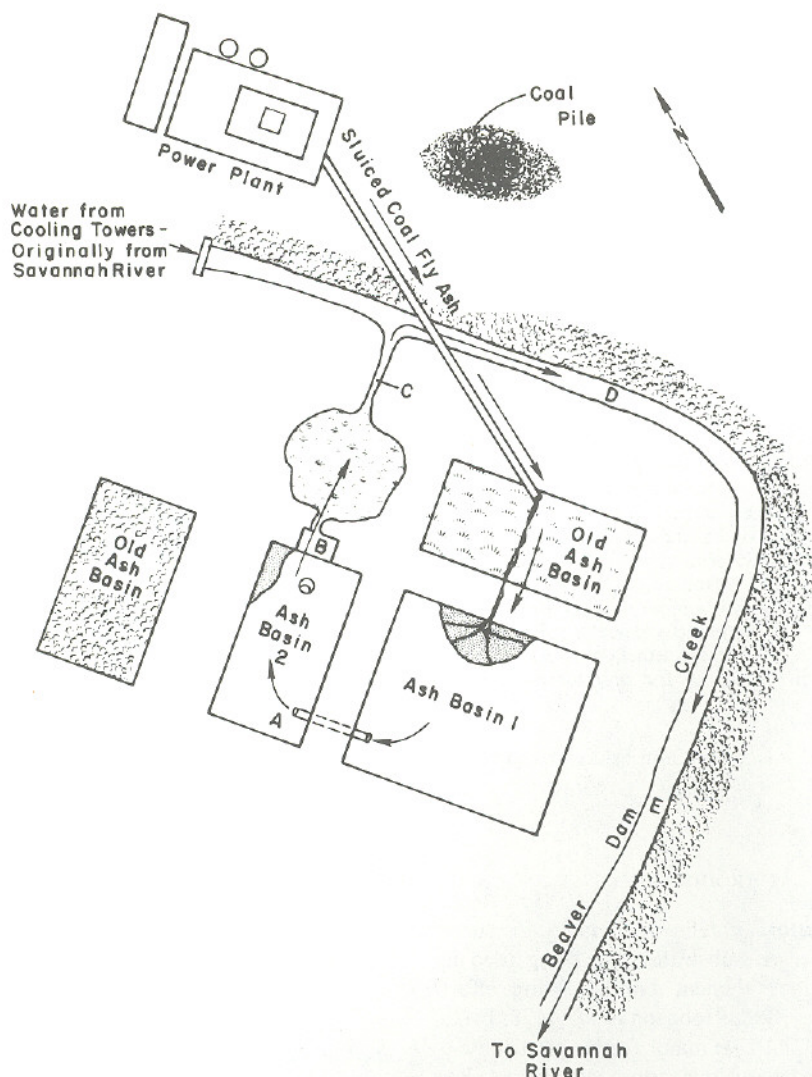
Fig. 1. Sample sites (A–E) associated with the 400D Area coal power plant. Another site (F) is located in Par Pond, a 1100 ha reservoir located approx. 20 km northeast of these sites.

were fastened within each PVC frame. Two frames and associated slides were placed at each site 6 weeks (September 1981) before sampling began.

Sampling was initiated in November 1981 and continued at 6 week intervals until July 1982. At each of the seven sampling dates, twenty-one slides were randomly removed from each site and placed into plastic slide boxes filled with sample site water. Within 3 h, the slides were processed. Material from three randomly chosen sides was scraped from the slides into individual vials containing Lugol's fixative. Cell density for each triplicate was determined using a Sedgwick-Rafter cell as outlined in *Standard Methods* (APHA, 1975). Material from six slides was then scraped into three ashed and tared crucibles (two slides each) for dry and ash weight determinations (APHA, 1975). Material on the remaining slides was scraped onto three Teflon™ watch glasses, dried at 85°C and analyzed as described below for arsenic, cadmium, chromium, copper, iron, manganese and zinc.

Water

At each sampling, a Hydrolab™ System 8000 *In Situ* Water Quality Analyzer was used to determine dissolved oxygen content, conductivity, temperature, oxidation-reduction potential and pH of the sampling site waters. All probe determinations were done in triplicate. Three water samples were also taken at each site using nine liter, linear polyethylene bottles. Within 2 h, 100 ml aliquots of the water samples were filtered through 0.45 μm Millipore™ Type HA filters and acidified with 0.5 ml of Teflon™ distilled nitric acid. All glassware and plasticware were acid soaked (50% v/v concentrated nitric acid) at least 2 days and then thoroughly rinsed with deionized/Milli-Q™ water before use. During the filtration process, a filtered sample water rinse was also performed. Procedural blanks were generated by passing deionized/Milli-Q™ water through the above process. These preserved samples were later analyzed for dissolved (filterable) arsenic, cadmium, calcium, chromium, copper, iron, magnesium, manganese and zinc.

Aliquots of the water samples were also analyzed for total alkalinity (potentiometric titration), sulfate (barium chloride turbidometric method) and chloride (silver nitrate titration) (APHA, 1975). Suspended solids were collected from approx. 8 l. of each sample using a Sorvall™ SS-3 Superspeed centrifuge equipped with a "Szent-Gyorgyi and Blum" KSB continuous flow system. Titanium tubes were used to reduce contamination. The system was operated at 10,000 rpm and a flow rate of 125 ml min⁻¹. These operating
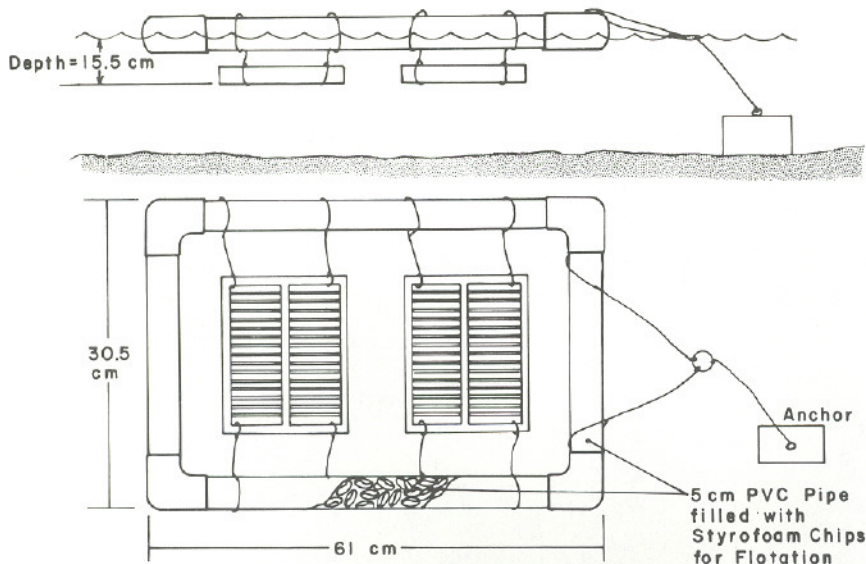
Fig. 2. *Aufwuchs* substrate racks with no metallic components. The PVC frames were filled with styrofoam chips and sealed with commercially available PVC cement. The PVC frames were designed to provide buoyancy and protection of the slides from any large objects floating downstream into the slide racks.

parameters allow the removal of particles greater than $0.16 \mu m$ in dia (spherical particle with a density of $2.65 \mathrm{g\,cm^{-3}}$). The suspended solids were dried (85°C), digested and analyzed for arsenic, cadmium, chromium, copper, iron, manganese and zinc as described below.

Elemental analyses

Dried *aufwuchs* or suspended solids samples were weighed, placed into Teflon™ beakers with 5 ml of Teflon™ distilled nitric acid, covered with a Teflon™ watch glass and digested for 6 h at 60–65°C. The samples were allowed to cool, brought to 25 ml volume with deionized/Milli-Q™ water and stored in 60 ml linear polyethylene bottles until analysis. All glassware and plasticware were acid-soaked and deionized/Milli-Q™ water rinsed as described previously. Procedural blanks consisted of beakers containing 5 ml of Teflon™ distilled acid but no sample, which were processed alongside the samples.

An Hitachi Model 180-70 Zeeman Effect atomic absorption spectrophotometer equipped with a GA-3 graphite atomizer was used to analyze the water, *aufwuchs* and suspended solids samples. Argon was used as a flow gas and standard conditions were used for the determinations of all elements except arsenic. Zirconium acetate-treated, pyrolytically-coated cuvettes (Vickrey *et al.*, 1980, 1981) and nickel nitrate addition (Ediger, 1975) were used to improve the arsenic analyses. Matrix effects for all elements were corrected by the methods of standard additions. Dissolved magnesium was determined in an air–acetylene flame while dissolved calcium was determined in a nitrous oxide–acetylene flame after lanthanum chloride addition.

Scanning electron microscopy

Slides were collected from the six sites in April 1982 for scanning electron microscopy and X-ray elemental analysis. The preparation methods and analytical parameters are described in detail elsewhere (Newman *et al.*, 1983). Briefly, the material was freeze-dried, mounted on spectroscopically pure, fused carbon planchets and sputter coated with carbon (15 min at 12 mA). An ETEC Autoscan scanning electron microscope equipped with a Canberra Model 8100 energy dispersive X-ray spectrometer was used to examine the

material coating the slides. The microscope was operated at 20 kV, with a $150 \mu m$ aperature, 12 mm working distance, 45° tilt angle and a fixed condensor current for all samples. A collection time of approximately fifteen minutes was used for each sample area examined.

The counts attributable to iron in the combined $Fe_{k\alpha}$ and $Mn_{k\beta}$ peak were ascertained by difference using the ratio of the counts in the $Mn_{k\alpha}$ and $Mn_{k\beta}$ peaks for spectra derived from samples with no detectable iron. The derived ratio agreed closely with published values (White and Johnson, 1970). No integrated counts were determined if a peak was detectable yet close to the background level. In such a case the element was noted as present only.

Statistical analysis

Partial correlation coefficients were calculated for the monitoring data using the GLM procedure of the SAS Statistical Package (Helwig and Council, 1979). Correlations were computed using the matrix of residual sums-of-squares and cross-products after removing the main effects of site (Morrison, 1967). The copper data for the April 1982 Beaver Dam Creek samples (sites D and E) were omitted from statistical consideration. On this date, a cooling tower washdown into Beaver Dam Creek caused very high copper values. The metal concentrations associated with the suspended solids were transformed to $\mu g\,l^{-1}$ of water prior to use in the statistical analysis.

RESULTS

General

Table 1 summarizes the water characteristics at the six sites. A detailed analysis of the water chemistry during the sampling period has been reported elsewhere (Alberts *et al.*, In press). The sites above Beaver Dam Creek (A–C) displayed the highest specific conductance, sulfate, arsenic and calcium concentrations of all the sites. The Beaver Dam Creek

Table 1. Water characteristics—overall summary by site

Variable	Site					
	A ($N = 21$)	B ($N = 21$)	C ($N = 21$)	D ($N = 21$)	E ($N = 21$)	F ($N = 21$)
Suspended solids ($mg\,l^{-1}$)	4.4 ± 3.1	2.6 ± 1.5	2.4 ± 1.2	16.1 ± 11.3	16.4 ± 7.9	9.4 ± 11.1
Temperature (°C)	19.7 ± 7.6	19.5 ± 7.4	20.1 ± 7.7	26.1 ± 5.4	26.4 ± 5.1	19.9 ± 7.2
pH	6.73 ± 0.38	6.82 ± 0.26	6.89 ± 0.28	6.69 ± 0.20	6.72 ± 0.22	7.34 ± 0.75
Dissolved oxygen ($mg\,O_2\,l^{-1}$)	9.60 ± 2.14	9.87 ± 1.89	9.90 ± 1.69	8.14 ± 1.19	8.50 ± 1.41	9.38 ± 0.63
Specific conductance ($mmhos\,cm^{-1}$)	0.109 ± 0.029	0.111 ± 0.026	0.115 ± 0.025	0.083 ± 0.010	0.081 ± 0.013	0.063 ± 0.009
Redox potential (mV)	135 ± 18	136 ± 20	130 ± 14	86 ± 37	114 ± 29	110 ± 23
Chloride ($mg\,l^{-1}$)	3.48 ± 0.77	3.37 ± 0.77	3.53 ± 0.70	3.28 ± 0.75	3.45 ± 0.68	3.48 ± 0.58
Total alkalinity ($mg\,l^{-1}$ as $CaCo_3$)	8.50 ± 3.10	8.31 ± 3.16	8.57 ± 3.13	16.35 ± 1.43	16.13 ± 1.37	13.85 ± 1.40
Sulfates ($mg\,l^{-1}$)	41.2 ± 7.8	41.6 ± 7.0	42.2 ± 6.3	10.6 ± 3.1	10.5 ± 2.8	4.9 ± 0.7
Dissolved elemental concentration ($mg\,l^{-1}$)						
Fe	22 ± 15	25 ± 21	25 ± 14	345 ± 134	315 ± 141	17 ± 8
Mn	27 ± 22	25 ± 21	30 ± 17	86 ± 45	82 ± 44	3 ± 5
As	48.9 ± 32.3	46.3 ± 30.4	43.9 ± 30.7	2.5 ± 2.0	2.2 ± 1.7	1.3 ± 1.6
Cd	0.38 ± 0.17	0.39 ± 0.30	0.38 ± 0.37	0.21 ± 0.17	0.23 ± 0.27	0.07 ± 0.07
Cr	0.50 ± 0.55	0.45 ± 0.47	0.31 ± 0.35	0.35 ± 0.31	0.38 ± 0.28	0.05
Cu	2.6 ± 0.6	2.6 ± 0.7	2.6 ± 0.9	$3.0 \pm 0.9*$	$3.0 \pm 1.0*$	0.8 ± 0.9
Zn	9 ± 6	8 ± 4	8 ± 7	12 ± 17	10 ± 16	2 ± 4
Mg	1733 ± 228	1723 ± 228	1726 ± 241	1333 ± 180	1332 ± 188	1070 ± 111
Ca	$12,093 \pm 1875$	$11,917 \pm 2202$	$12,210 \pm 2731$	4540 ± 356	4712 ± 284	3750 ± 374

Mean $\pm$ 1 SD.
*After omission of the April 1982 samples ($N = 18$).

Sites (D and E) had the highest suspended solids load, temperatures and iron concentrations. The waters of Beaver Dam Creek are pumped from the Savannah River to the power plant principally for cooling purposes. After leaving the power plant, the warm water is pumped into a ditch to form the head of Beaver Dam Creek. The rapidly flowing, warm waters carry suspended solids originating from the stream bottom (resuspension) and the Savannah River. A general decrease is indicated for arsenic, cadmium and chromium with distance away from the ash basins. The Par Pond water had the lowest dissolved concentrations for all these elements.

Table 2 is a summary of the suspended solids characteristics at the six sites. The ash basin suspended solids appeared to be principally ash and plankton. Occasional algal blooms gave the material a green/grey color. The majority of the material at sites B and C was of similar composition. The trace element content in the suspended solids seemed to increase slightly with distance downstream until it entered Beaver Dam Creek. However, dilution by the high levels of relatively uncontaminated suspended solids of Beaver Dam Creek caused a decrease in the suspended solids associated concentrations of arsenic, cadmium, copper and zinc. Iron concentrations increased in the suspended solids while manganese concentrations remained at approximately the same level. As the major portion of the suspended solids in Beaver Dam Creek appeared to be a red clay and the dissolved iron concentrations in this creek were high, this observation is not surprising. The trace element concentrations, with the exception of manganese, were quite low in the Par Pond suspended solids. The suspended solids of Par Pond appeared to be comprised of plankton, plant fragments and, occasionally, small amounts of fine sand from a nearby shoal.

The characteristics of the procedurally-defined *aufwuchs* from the six sites are given in Table 3. The material accumulating on the slides from the ash basin (site A) had the lowest per cent ash-free weight (an indication of the amount of organic material associated with the slides) and moderate cell densities. More material was found on the slides below the ash basins (sites B and C). This material had high cell densities as well as high per cent ash-free weight values. The large amount of material accumulating on the Beaver Dam Creek slides (sites D and E) had considerably less cells and organic material (as indicated by the per cent ash-free weight) than any of the non-ash basin sites. Despite the small amount of material on the Par Pond slides (site F), the cell densities were high and the per cent ash-free weight values indicated that a large percentage of the material was organic matter. Iron and manganese concentrations varied considerably between sites with the Beaver Dam Creek sites having high iron concentrations. The arsenic, cadmium, copper and zinc concentrations in the material from sites A through

C were high relative to the other sites. Chromium, however, increased slightly in the Beaver Dam Creek materials relative to the upstream sites.

Correlation analyses

The results of the correlation analyses are given in Table 4. For the three elements, cadmium, copper and zinc, positive correlations were seen between the elemental concentrations in the suspended solids and those in the procedurally-defined *aufwuchs*. With the exception of chromium, extremely strong, positive correlations were noted between the manganese concentrations associated with the procedurally-defined *aufwuchs* and the elemental concentrations (i.e. As, Cd, Cu or Zn) of this material. Arsenic, chromium and copper were positively correlated with the iron concentrations associated with the procedurally-defined *aufwuchs*. Strong, negative correlations were noted for all five elements and the cell densities on the glass slides. Negative (As, Cr), weak (Cd) or nonsignificant (Cu, Zn) correlations were noted between the elemental concentrations in the material and the per cent ash-free weight of the material.

Scanning electron microscopy

Typical areas on the slides are shown in Fig. 3. Calculated cell densities (cells mm^{-2}) for slides were: A–70, B–8, 640, C–90, D–310, E–1720 and F–234. Despite these cell densities, the majority of the material coating slides from sites A, C, D and E was abiotic in nature. The encrusting material particularly on the site C slides had a characteristic appearance described earlier by Newman *et al.* (1983) (amorphous coating with scattered and raised pocks). Material on site A slides consisted largely of fly ash while those of site B were a mixture of diatoms and encrusting matrix. Material from the Par Pond site appeared to consist of diatoms and organic debris.

X-ray spectra were collected from areas on slides from all sites except site D (Table 5). The fly ash and matrix on the slides from sites A–C were high in manganese and iron. The Beaver Dam Creek material was also high in iron but no manganese was detected with this technique. For a particular site, these two elements were consistently lower in diatoms relative to the matrix (or fly ash). It should be understood that the values for diatoms include a partial X-ray contribution from surrounding materials. No matrix was found on the Par Pond slides. Spectra collected from an area devoid of diatoms on the Par Pond slide surface gave spectra typical of glass. Little iron and manganese were detected. Cadmium was not detected in any of the areas examined. When detected, arsenic, chromium, copper and zinc were consistently higher in abiotic components than biotic components of the material.

DISCUSSION

The nature and chemical composition of the material accumulating on the slides (and procedurally

Table 2. Suspended solids characteristics—overall summary by site

	Site					
Variable	A ($N = 21$)	B ($N = 21$)	C ($N = 21$)	D ($N = 21$)	E ($N = 21$)	F ($N = 21$)
Suspended solids ($mg\,l^{-1}$)	4.4 ± 3.1	2.6 ± 1.5	2.4 ± 1.2	16.1 ± 11.3	16.4 ± 7.9	9.4 ± 11.1
Elemental concentration ($\mu g\,g^{-1}$ dry wt)						
Fe	$21{,}089 \pm 5623$	$24{,}344 \pm 4886$	$27{,}782 \pm 9095$	$38{,}981 \pm 10{,}943$	$39{,}533 \pm 17{,}298$	$13{,}558 \pm 9737$
Mn	3397 ± 2574	4469 ± 3445	5613 ± 4621	3848 ± 2301	3063 ± 1373	6544 ± 4609
As	644 ± 304	774 ± 251	870 ± 456	34 ± 28	23 ± 13	8 ± 18
Cd	8.1 ± 5.3	10.6 ± 8.2	10.2 ± 8.5	0.9 ± 0.4	1.0 ± 0.7	0.9 ± 0.7
Cr	64.3 ± 26.8	79.2 ± 26.3	77.0 ± 24.0	49.8 ± 15.0	54.3 ± 18.6	11.4 ± 6.7
Cu	176 ± 75	227 ± 251	219 ± 99	96 ± 76*	104 ± 82*	30 ± 23
Zn	368 ± 89	492 ± 224	444 ± 204	255 ± 235	234 ± 211	131 ± 151

Mean ± 1 SD.
*After omission of the April 1982 samples ($N = 18$).

Table 3. *Aufwuchs* characteristics—overall summary by site

	Site					
Variable	A ($N = 21$)	B ($N = 21$)	C ($N = 21$)	D ($N = 21$)	E ($N = 21$)	F ($N = 21$)
Cell density (cells mm^{-2})	432 ± 903	8962 ± 6941	4498 ± 5292	132 ± 105	707 ± 1010	1888 ± 2145
Dry weight ($g\,m^{-2}$)	2.9 ± 3.4	6.5 ± 4.3	7.3 ± 11.3	12.1 ± 6.8	3.7 ± 2.8	1.7 ± 1.5
% Ash-free weight	19 ± 13	41 ± 11	41 ± 11	23 ± 15	29 ± 10	54 ± 9
Elemental concentration ($\mu g\,g^{-1}$ dry wt)						
Fe	$11{,}428 \pm 4858$	9880 ± 8282	$13{,}597 \pm 6515$	$94{,}928 \pm 81{,}595$	$94{,}404 \pm 57{,}671$	9590 ± 6282
Mn	$11{,}638 \pm 9126$	$23{,}639 \pm 22{,}440$	$20{,}152 \pm 25{,}528$	5045 ± 5108	$10{,}311 \pm 14{,}542$	7603 ± 3674
As	375 ± 201	452 ± 342	677 ± 346	136 ± 82	153 ± 70	6 ± 8
Cd	25.5 ± 20.5	39.4 ± 27.0	31.3 ± 30.4	1.0 ± 0.5	2.3 ± 2.0	2.1 ± 2.8
Cr	39 ± 16	35 ± 27	35 ± 21	43 ± 22	67 ± 14	5 ± 2
Cu	232 ± 130	294 ± 163	161 ± 92	80 ± 72*	120 ± 76*	20 ± 23

Table 4. Partial correlation coefficients after removal of site effects (d.f. $= 112$)

Variable	Arsenic	Chromium	Cadmium	Copper	Zinc
			Element		
Dissolved elemental concentration	-0.087	-0.528§	-0.149	0.178	0.186*
Elemental concentration in suspended solids	0.053	0.002	0.297‡	0.367§	0.210*
Manganese concentration in *aufwuchs*	0.431§	-0.065	0.832§	0.826§	0.928§
Iron concentration in *aufwuchs*	0.185*	0.275†	-0.021	0.280‡	0.019
Cell density	-0.523§	-0.317‡	-0.451§	-0.463§	-0.485§
% Ash-free weight	-0.277†	-0.522§	0.179*	0.006	0.120

*Significant at $\alpha = 0.05$.
†Significant at $\alpha = 0.01$.
‡Significant at $\alpha = 0.001$.
§Significant at $\alpha = 0.0001$.

defined as *aufwuchs*) varied considerably between sites. However, materials from all but the Par Pond site were rich in manganese or iron. A large portion of this material was abiotic in nature. An abiotic, ferromanganese matrix similar to that described earlier on slides submerged in two New Jersey reservoirs (Newman *et al.*, 1982) was present on slides from sites B through E. Material on site A slides was also predominantly abiotic, however, ash particles were the major constituent of the material. The majority of the material on Par Pond slides was composed of algal clusters and entrapped organic debris. The X-ray analyses suggest that the major portion of the five trace elements of interest is associated with the abiotic matrix or ash, not the microflora.

The correlation analyses strongly suggest that the major portion of the five elements are associated with abiotic components of the material coating the slides. The positive correlations between concentrations of the five elements and iron or manganese concentrations in the material indicate that hydrous manganese oxides, and, perhaps, iron oxides may be the major factors controlling the realized concentrations of these five trace elements in the procedurally-defined *aufwuchs*. Hydrous iron and manganese oxides play a dominant role in the incorporation of trace elements into solid phases within aquatic systems, i.e. suspended solids (Crecelius, 1975) and sediments (Jenne, 1968), therefore, this suggestion that hydrous iron and manganese oxides play a dominant role in trace element accumulation in the procedurally-defined *aufwuchs* has sufficient precedence. Large coefficients were noted between *aufwuchs*-associated manganese and *aufwuchs*-associated cadmium (0.837), copper (0.826) and zinc (0.928) while smaller coefficients were noted between *aufwuchs*-associated manganese and *aufwuchs*-associated chromium (-0.066) and arsenic (0.431). Also, *aufwuchs*-associated cadmium and zinc displayed nonsignificant correlations with *aufwuchs*-associated iron.

Initial interpretation of this results can be made using the pH of zero charge (pH_{zpc}) of manganese and iron oxides. Except for βMnO_2, manganese oxides have pH_{zpc} values ranging from 1.5 to 5.6 (Kinniburgh and Jackson, 1981; Stumm and Morgan, 1981). Values for iron oxides vary from 6.5 to 8.7 (Kinniburgh and Jackson, 1981; Stumm and Morgan, 1981). Therefore, at the pH conditions prevalent in the systems studied, the manganese oxides will have a net negative surface charge while the iron oxides would be expected to possess a net positive surface charge. Hence, dissolved cadmium and zinc, present as hydrated cations, should be readily adsorbed to the manganese oxides. Copper, present as a hydrated cation or a carbonato complex, would also adsorb to the manganese oxides. Arsenic, present as an oxyanion, should adsorb to the iron oxides. However, arsenic also appears to be correlated with *aufwuchs* associated manganese. Chromium was predicted to be present predominantly as $CrCO_3^+$ (GEOCHEM, Mattigod and Sposito, 1979), however, it was correlated with *aufwuchs*-associated iron, not manganese. As specific adsorption can occur under the influence of non-ionic and ionic bonding, the adsorption behaviors of chromium and arsenic are not implausible (Pierce and Moore, 1982). Coprecipitation (Aoki and Munemori, 1982), organic complex formation (Nakayama *et al.*, 1981) and other factors (Jenne, 1968; Davis and Leckie, 1978) could contribute to these results.

Although algae accumulate high levels of trace elements (Ray and White, 1976; Trollope and Evans, 1976; Patrick, 1978), the negative correlations between cell density and elemental concentrations indicate that the role of hydrous oxides overshadows that of bioaccumulation by the microflora. Indeed, increased algal cell densities appear to decrease the amount of hydrous oxides per unit of mass available to bind the trace elements and, as a result, indirectly decrease the amount of trace element binding to the material.
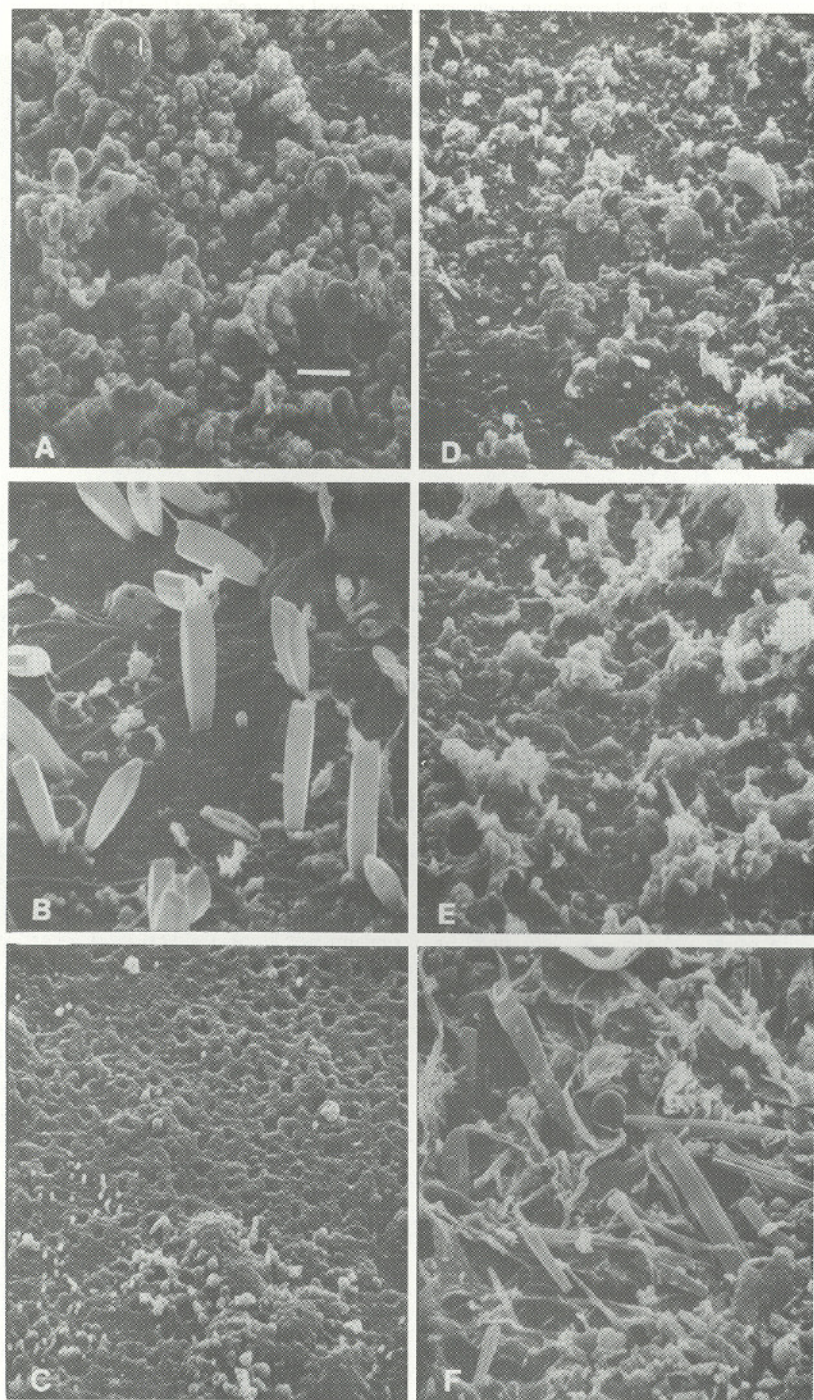
Fig. 3. Scanning electron micrographs of material accumulating on the glass slides. The micrographs were taken from typical areas of slides sampled April 1982 from the six sites. The sites are labelled A–F in the lower left-hand corner of each frame. The white bar in the lower right-hand corner of micrograph A indicates 10 μm. All micrographs were taken at the same magnification (1400 $\times$).

The results of this study support those of earlier work with lead in procedurally-defined *aufwuchs* from two New Jersey reservoirs (Newman *et al.*, 1983) and impart some generality to the hypothesis that hydrous iron and manganese oxides may play a dominant role in determining the trace element concentrations in procedurally-defined *aufwuchs*. This suggests the potential for generating invalid bioaccumulation data using procedurally-defined *aufwuchs*. Additionally, the data indicate the need for

Table 4. Partial correlation coefficients after removal of site effects (d.f. = 112)

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defined as *aufwuchs*) varied considerably between sites. However, materials from all but the Par Pond site were rich in manganese or iron. A large portion of this material was abiotic in nature. An abiotic, ferromanganese matrix similar to that described earlier on slides submerged in two New Jersey reservoirs (Newman *et al.*, 1982) was present on slides from sites B through E. Material on site A slides was also predominantly abiotic, however, ash particles were the major constituent of the material. The majority of the material on Par Pond slides was composed of algal clusters and entrapped organic debris. The X-ray analyses suggest that the major portion of the five trace elements of interest is associated with the abiotic matrix or ash, not the microflora.

The correlation analyses strongly suggest that the major portion of the five elements are associated with abiotic components of the material coating the slides. The positive correlations between concentrations of the five elements and iron or manganese concentrations in the material indicate that hydrous manganese oxides, and, perhaps, iron oxides may be the major factors controlling the realized concentrations of these five trace elements in the procedurally-defined *aufwuchs*. Hydrous iron and manganese oxides play a dominant role in the incorporation of trace elements into solid phases within aquatic systems, i.e. suspended solids (Crecelius, 1975) and sediments (Jenne, 1968), therefore, this suggestion that hydrous iron and manganese oxides play a dominant role in trace element accumulation in the procedurally-defined *aufwuchs* has sufficient precedence. Large coefficients were noted between *aufwuchs*-associated manganese and *aufwuchs*-associated cadmium (0.837), copper (0.826) and zinc (0.928) while smaller coefficients were noted between *aufwuchs*-associated manganese and *aufwuchs*-associated chromium (−0.066) and arsenic (0.431). Also, *aufwuchs*-associated cadmium and zinc displayed nonsignificant correlations with *aufwuchs*-associated iron.

Initial interpretation of this results can be made using the pH of zero charge (pH$_{zpc}$) of manganese and iron oxides. Except for βMnO_2, manganese oxides have pH$_{zpc}$ values ranging from 1.5 to 5.6 (Kinniburgh and Jackson, 1981; Stumm and Morgan, 1981). Values for iron oxides vary from 6.5 to 8.7 (Kinniburgh and Jackson, 1981; Stumm and Morgan, 1981). Therefore, at the pH conditions prevalent in the systems studied, the manganese oxides will have a net negative surface charge while the iron oxides would be expected to possess a net positive surface charge. Hence, dissolved cadmium and zinc, present as hydrated cations, should be readily adsorbed to the manganese oxides. Copper, present as a hydrated cation or a carbonato complex, would also adsorb to the manganese oxides. Arsenic, present as an oxyanion, should adsorb to the iron oxides. However, arsenic also appears to be correlated with *aufwuchs* associated manganese. Chromium was predicted to be present predominantly as $CrCO_3^+$ (GEOCHEM, Mattigod and Sposito, 1979), however, it was correlated with *aufwuchs*-associated iron, not manganese. As specific adsorption can occur under the influence of non-ionic and ionic bonding, the adsorption behaviors of chromium and arsenic are not implausible (Pierce and Moore, 1982). Coprecipitation (Aoki and Munemori, 1982), organic complex formation (Nakayama *et al.*, 1981) and other factors (Jenne, 1968; Davis and Leckie, 1978) could contribute to these results.

Although algae accumulate high levels of trace elements (Ray and White, 1976; Trollope and Evans, 1976; Patrick, 1978), the negative correlations between cell density and elemental concentrations indicate that the role of hydrous oxides overshadows that of bioaccumulation by the microflora. Indeed, increased algal cell densities appear to decrease the amount of hydrous oxides per unit of mass available to bind the trace elements and, as a result, indirectly decrease the amount of trace element binding to the material.

Table 5. X-ray data from selected areas of material coating the slides

Site	Sample area	Counts collected after 15 min of X-ray collection						
		Fe	Mn	As	Cr	Cu	Cd	Zn
A	Fly Ash	$42{,}482 \pm 206$	$310{,}956 \pm 558$	ND	ND	Present	ND	1772 ± 42
	Diatom	$14{,}717 \pm 121$	3328 ± 58	ND	ND	ND	ND	ND
B	Matrix	6284 ± 79	$19{,}073 \pm 138$	ND	ND	ND	ND	ND
	Diatom	5172 ± 72	$11{,}801 \pm 109$	ND	ND	ND	ND	ND
C	Matrix	$18{,}744 \pm 137$	$179{,}016 \pm 423$	Present	ND	ND	ND	1488 ± 39
	Diatom	$13{,}192 \pm 115$	6905 ± 83	ND	ND	ND	ND	ND
E	Matrix	$177{,}060 \pm 421$	ND	ND	ND	3537 ± 59	ND	ND
	Matrix	$244{,}973 \pm 474$	ND	ND	2802 ± 53	3266 ± 40	ND	ND
	Diatom	$121{,}257 \pm 348$	ND	ND	ND	1574 ± 40	ND	ND
F	Diatom (No matrix)	5338 ± 73	2146 ± 46	ND	ND	ND	ND	ND

Mean $\pm$ 1 SD.
ND—none detected with this method.

closer examination of the bioavailability to grazing organisms of the highly concentrated trace contaminants in materials coating submerged surfaces.

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