JA7

JA-87

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1977, p. 89-96

Vol. 33, No. 1
Printed in U.S.A.

Effects of Naturally Occurring Aquatic Organic Fractions on ^{241}Am Uptake by *Scenedesmus obliquus* (*Chlorophyceae*) and *Aeromonas hydrophila* (*Pseudomonadaceae*)

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Received for publication 26 May 1976

Naturally occurring organics were extracted from water collected from Skinface Pond near Aiken, S.C. Organics were separated into four nominal diameter size fractions (I, >0.0183; II, 0.0183 to 0.0032; III, 0.0032 to 0.0009; IV, <0.0009 μm) by membrane ultrafiltration and introduced into *Scenedesmus obliquus* and *Aeromonas hydrophila* cultures to determine their effects on ^{241}Am availability for uptake. Effects on ^{241}Am uptake were determined in actively growing *S. obliquus* cultures after 96 h of growth and in dense cultures of nongrowing cells after 4 h. Uptake by *A. hydrophila* was determined after 4 and 24 h in actively growing cultures. All organic fractions stimulated *S. obliquus* growth, with the most pronounced effects due to larger organic fractions, whereas no apparent growth stimulation of *A. hydrophila* was observed for any organic fraction. For both long-term and short-term studies, cellular ^{241}Am concentration (picocuries/cell) increased with increasing ^{241}Am concentration for *S. obliquus* and *A. hydrophila*. Fraction IV increased ^{241}Am uptake by both *S. obliquus* and *A. hydrophila* during 4-h incubations. During 96-h incubations fraction I was flocculated and cosedimented, with *S. obliquus* and *A. hydrophila* cells causing an apparent increase in ^{241}Am uptake. Fractions II and III reduced apparent ^{241}Am uptake by *S. obliquus* as a result of biological dilution caused by increased algal growth due to the organics. Fraction IV caused a reduction in ^{241}Am uptake by *S. obliquus* not attributable to biological dilution. Organics increased ^{241}Am uptake by *A. hydrophila* during 4- and 24-h incubations. *A. hydrophila* also caused flocculation of fraction I during 96-h incubations.

The transplutonic actinide ^{241}Am, produced by decay of the fission product ^{241}Pu, has gained importance as an environmental contaminant due, in part, to its higher specific activity and increased mobility relative to plutonium in soil systems and its increased uptake relative to plutonium by biota in terrestrial ecosystems (32, 43; A. Wallace and M. Romney, presented at the Annual Meeting of the American Society of Agronomy, Knoxville, Tenn., 24–29 August 1975). Americium is considered to be among the most toxic elements known to man (10). Releases of this potentially hazardous material from nuclear weapons and power industries are expected to increase with the advent of the liquid-metal fast-breeder reactor.

Information concerning the behavior of ^{241}Am in aquatic ecosystems is not replete. Amory et al. (11) studied ^{241}Am in a contaminated pond system, and ^{241}Am is known to be concentrated by algae and bacteria under laboratory conditions (15). Since algae and bacteria are important in the transport and mobilization of elements, information on factors affecting ^{241}Am uptake is needed to predict the fate of transplutonic elements released into the environment. Cycling processes and biological uptake of ^{241}Am must be understood prior to environmental releases so that rational assessments of its hazard can be determined and standards evaluated.

Francis (12) has stated that chelation with naturally occurring organic soil components may be important in movement of transuranics in natural food chains leading to humans. Szalay (48) suggested that humic acids be used in the disposal of radioactive wastes because these organic compounds immobilize radionuclides, thus combating water pollution. Although Bondietti et al. (6) have investigated Pu-humic interactions, no studies have considered the effect of humics on the availability of transuranic actinides to biological systems. Recalcitrant, naturally occurring organic compounds, var-

iously known as humics, fulvics, and tannics, are of worldwide distribution in soil and aquatic systems. Because of their ubiquity, these large polyphenolic compounds are involved in chemical processes of nearly all surface waters (40). Organic ligands, which can form complexes and chelates with metals, are important in determining the form, movement, and availability of trace metals in natural waters (1, 14, 21, 22, 26, 28, 36).

The surface waters of the southeastern United States are soft and contain high concentrations of refractory organic compounds. This, coupled with the large number of present and projected nuclear power plants and nuclear fuel production and reprocessing facilities for the region, makes crucial the understanding of ^{241}Am cycling and fluxing processes in aquatic ecosystems. The objective of this study was to explore the effects of naturally occurring organics on ^{241}Am uptake by an algal and bacterial species.

MATERIALS AND METHODS

Ten-liter water samples were collected in polyethylene carboys from Skinface Pond, Aiken County, S. C., on 15 January 1976. Particulates of nominal diameter ≥ 0.15 μm were removed using a Sorvall SS-1 centrifuge equipped with a KSB continuous flow system (Sorvall, Norwalk, Conn.). Centrifuged water was passed sequentially through XM-300, PM-10, and UM-05 Diaflo ultrafilters under an N_2 atmosphere using a model 402 stirred cell (Amicon Co., Lexington, Mass.). This resulted in four nominal diameter fractions, three of which were retained by ultrafilters and one which passed all four filters (see Table 1).

The maximum attainable shear across the membrane was maintained using the stirred cell. Flocculation was minimized by removing retained organics from the cell frequently and maintaining water over the cell at all times. Each fraction was washed three times by resuspending the fraction retained by each filter in distilled HOH and refiltering. Each wash was then added to the residual solution before the next fractionation. New filters were prepared using methods recommended by the manufacturers and washed in distilled water. These procedures eliminated contamination due to the ultrafilters. Total organic carbon in each fraction was determined using a Beckman model 915 carbon analyzer.

Axenic cultures of *S. obliquus* (Türp) Kütz (strain no. 1592) were obtained from the Indiana University Culture Collection (46). *Scenedesmus* species are ubiquitous, occurring in almost every freshwater environment (5). This genus may make up as much as 90% of some phytoplankton communities (37) and is common in softwater ponds containing high concentrations of humic substances (25). *A hydrophila* (strain no. 7966), obtained from the American Type Culture Collection, was chosen for study because it is common in the sediments and water column of ponds and lakes (49–50).

Stock, axenic *S. obliquus* cultures were maintained in 200 ml of algal assay procedure (AAP) medium aerated with sterile air (2). Stock algal cultures were checked periodically for bacterial contamination by plating on peptose agar, incubating in tryptic soy broth (TSB), and microscope examination. Stock and experimental cultures were incubated at 24 ± 2°C in a controlled environment chamber with 4,035-lux illumination from balanced spectrum Growlux fluorescent bulbs on a 16 h of light-8 h of dark regime. Algal inocula were taken from 10-day-old *S. obliquus* cultures with cell densities of 5×10^5 cells/ml and relative growth rate (k') of 0.59/day. Initial *S. obliquus* cell densities were diluted to 5×10^4 cells/ml in 96-h studies and concentrated by centrifugation to 5×10^5 cells/ml in 4-h studies. Stock *A. hydrophila* cultures were maintained in AAP-TSB medium (Difco). *A. hydrophila* inocula were drawn from 24-h cultures with cell densities of 5×10^6 cells/ml (k', 3.4/day; doubling time (G), 8.72×10^{-2}/day) to make an initial cell density of 5×10^5 cells/ml in experimental flasks. Algal uptake was studied in 4- and 96-h incubations, whereas bacteria were incubated for 4 and 24 h. Experimental cultures were agitated on a rotary shaker at 200 rpm. Algal and bacterial cell densities were determined using calibrated phytoplankton and Petroff-Hausser counting chambers, respectively.

^{241}Am uptake was determined in AAP medium for *S. obliquus* and in AAP plus 0.05% TSB for *A. hydrophila*. Organic fraction isolates were added to experimental media, rendering concentrations equal to the water from which they were extracted. To study organics passing the smallest ultrafilter, AAP nutrients and TSB were added to the filtrate and distilled water to make experimental and control media, respectively. ^{241}Am was added as the nitrate (10^{-5} N HNO_3) to give concentrations of 5×10^{-5} and 10^{-5} μCi/ml in short-term uptake studies and 10^{-4} and 10^{-5} μCi/ml in long-term studies. The resulting HNO_3 concentration in the experimental flask was 10^{-7} N.

TABLE 1. *Organic carbon distribution in ultrafilter fractionation ranges of Skinface Pond water*

Ultrafilter	Fraction	Nominal mol wt	Organic carbon (mg/liter)	Organic carbon (% total)
XM-300	(I) F[a] > 0.0183 μm	F > 300,000	2.2	7
PM-10	(II) 0.0183 > F > 0.0032 μm	300,000 > F > 10,000	3.8	12
UM-05	(III) 0.0032 > F > 0.0009 μm	10,000 > F > 500	11.1	36
UM-05	(IV) F > 0.0009 μm	F > 500	14.0	45

[a] F, organic fraction isolated.

Algae and bacteria were separated from experimental media by centrifugation into 28.5% (by weight) bis(2-ethylhexyl)sebacate in *N*-butyl phthalate. This mixture produces a low-viscosity organic separator with a density of 1.0003 g/cm^3 that is nonmiscible in aqueous solution (3). Portions (40 ml) of algal or bacterial cell suspensions were centrifuged with 4 ml of organic separator in 50-ml polyethylene centrifuge tubes at 10^4 rpm for 25 min. This separated cells from experimental medium without filtration, which can sorb ^{241}Am and organics. This procedure also eliminated washing procedures that may alter the amount of ^{241}Am associated with cells. The experimental medium was aspirated off, and cells were suspended in 10-cm glass vials with 5 ml of acetone. Control cultures, containing all components of the medium but no algal or bacterial cells, were handled in the same manner as experimental cultures to evaluate contamination in the separation procedure. Portions (5 ml) were drawn from each experimental flask and analyzed to determine ^{241}Am plating losses to glass flasks. Reagent blanks, controls, and experimentals were analyzed for ^{241}Am using a GeLi detector interfaced to an INOTEC 4000 channel pulse height analyzer. Minimum detectable activity was 18 pCi/sample for a 100-min counting time.

Long-term uptake studies (96 h) were completely randomized 2^3-factorial experiments with four replications per treatment. Short-term uptake studies (4 h) were completely randomized 5 × 2 factorials with two replications per treatment. Since all replicates could not be centrifuged concurrently, experimental units were blocked orthogonally over time to evaluate error introduced by this procedure. Factorial effect totals for 2^3 factorials were computed using Yates' automatic method, whereas effect totals for 5 × 2 factorials were directly computed (8). Significance of factorial main effects was tested using standard ANOVA techniques (8), and means were separated with Student-Newman Keuls multiple range test (45). Significance was tested for at the $\alpha = 0.05$ level.

RESULTS AND DISCUSSION

Organic carbon. Particle settling is dependent on particle density and diameter and fluid viscosity, which are related by the Svedberg coefficient. Particles with similar coefficients settle together. Assuming particles to have the density of kaolinite clay (2.66 g/cm^3), which is the dominant clay type in the southeastern United States, particles sedimented together using a rotor speed of 7,000 rpm and flow rate of 10.1 liter/h would be 0.15 μm in diameter. This diameter is often used as the boundary between colloids and particulates.

Membrane ultrafiltration has been used successfully to fractionate organic compounds from natural water (16–18, 39). Ultrafiltration methods may be used to concentrate organics from water and separate them into nominal-sized fractions without losses and chemical extractions. The nature of Diaflo ultrafilters theoretically minimizes membrane polarization. However, because of the high organic content of Skinface Pond water and the large volumes of water fractionated, the maximum obtainable shear obtained in the stirred cell was not sufficient to prevent membrane polarization and concomitant decrease in apparent membrane pore diameters. For this reason, each retained fraction was resuspended three times in distilled water and refiltered. Additional carbon passed the membrane with each washing, but the amount passing filters in the third volume was very small. The volume change in the fraction passing all three filters due to this washing procedure was minimal compared to the volume fractionated. Gjessing (16) also used washing to correct for changing pore size during fractionation.

Ultrafiltration resulted in some flocculation of organics on the XM-300 filter. This flocculation was dispersed using low-level sonification. Pilot studies showed that this did not detectably change the molecular diameter composition. Flocculation during ultrafiltration was not evident for the other fractions.

Total organic carbon in Skinface Pond was 31.1 mg/liter (Table 1), which is approximately four times the world average for rivers (13) and is typical of southeastern aquatic systems (4). More than 80% of the TOC comprised fractions III and IV (Table 1). These lower-molecular-weight organics are probably fulvic in nature (4).

Differential growth. Addition of each of the organic fractions to AAP medium stimulated *S. obliquus* growth over AAP alone (Fig. 1). After 96 h, *S. obliquus* standing crops were approximately four times greater in media containing organic fractions I, II, or III (Fig. 1). Fraction IV also stimulated algal growth, but to a lesser extent than the three larger fractions. *A. hydrophila* growth was not significantly affected by the presence of organics (Fig. 2). After 24 h there was no significant difference between standing crops of *A. hydrophila* in media with or without each of the organic fractions.

A number of investigators have observed increased algal growth rate and final standing crops in media containing humic substances (24, 29, 30, 41). Prát (31) and Giesy (14) reported humic acids stimulate *S. obliquus* population growth. Prakash et al. (30) also found the larger-molecular-weight fraction of naturally occurring colored organics to be more stimulatory to freshwater and marine phytoplankton than smaller fractions. Humic substances also

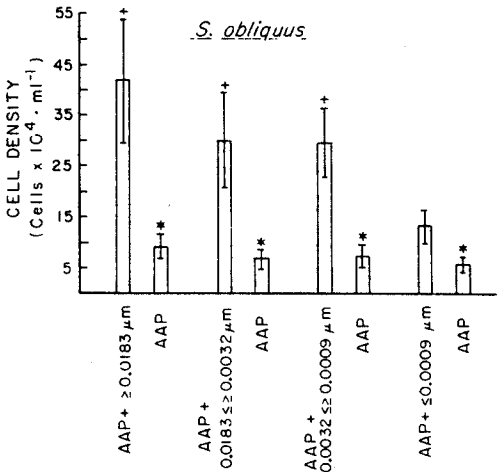

FIG. 1. *Effects of organic carbon fractions on S. obliquus standing crop after 96 h of growth in AAP medium. N = 4, α = 0.05, confidence intervals = $\pm 2S_{\bar{x}}$. Means that are not significantly different from each other are denoted by + or *.*

increase growth of bacteria such as *Pseudomonas* (9) and *Azotobacter* (7).

^{241}Am uptake. Uptake is herein defined as the association of ^{241}Am with algal or bacterial cells over time. Uptake does not imply an active mechanism, and no attempt was made to determine if ^{241}Am was associated with the surface or interior of the cell. Studies of live and fixed cells indicate that ^{241}Am uptake by phytoplankton is primarily a sorption phenomenon (15). Although there may be differences between surface-bound and internally bound availability for trophic transfer, these studies were designed only to determine the effects of naturally occurring organics on ^{241}Am uptake by an algal and bacterial species.

In short-term experiments (4 h) there was no algal population growth in any experimental treatment, eliminating this confounding effect. More ^{241}Am was taken up by *S. obliquus* at the higher ^{241}Am concentrations in AAP alone and in organic fractions III and IV (Table 2). Fraction I or II did not significantly affect ^{241}Am uptake by *S. obliquus* after 4 h at the high or low ^{241}Am medium concentration. Fraction III increased ^{241}Am uptake at the higher ^{241}Am level but did not effect uptake at the lower level. Fraction IV increased ^{241}Am uptake at both ^{241}Am medium concentrations, whereas fraction III increased ^{241}Am uptake only at the higher ^{241}Am medium concentration. Increased ^{241}Am uptake in the presence of fractions III and IV could be due to their smaller nominal molecular weight or the greater concentration of organics in these fractions.

^{241}Am uptake in long-term studies was consistently greater at the higher ^{241}Am concentration (Table 3). Fractions II, III, and IV all significantly reduced cellular ^{241}Am concentration as compared to AAP at both ^{241}Am medium concentrations. Fraction I, however, significantly increased cellular ^{241}Am concentration at both ^{241}Am medium concentrations. Reduced cellular ^{241}Am concentration in long-term studies, in the presence of organic fractions, indicates reduced uptake probably due to chelation. However, this reduction may be due to biological dilution. Giesy and Paine (15) found reduction of cellular ^{241}Am concentration due to biological dilution in *S. obliquus* and two other species of green algae. Each of the four organic fractions studied increased *S. obliquus* growth rate and standing crop after 96 h (Fig. 1 and Table 3) with a concomitant decrease in ^{241}Am concentration per cell (Table 3). Fraction IV significantly reduced ^{241}Am concentration per cell at the higher ^{241}Am level even though the

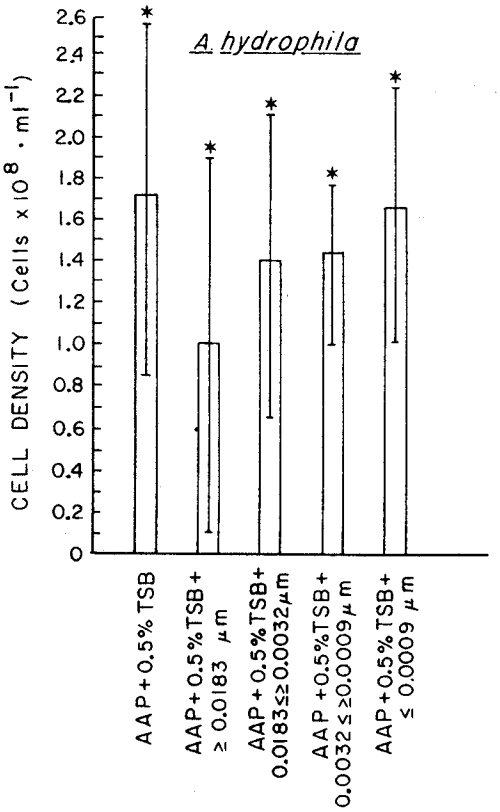

FIG. 2. *Effects of organic carbon fractions on A. hydrophila standing crop after 24 h of growth in AAP + 0.5% TSB medium. N = 4, α = 0.05, confidence intervals = $\pm 2S_{\bar{x}}$. Means that are not significantly different from each other are denoted by *.*

TABLE 2. *Effect of four organic fractions on S. obliquus cellular ^{241}Am concentration after 4 h*[a]

Fraction	^{241}Am/Cell (pCi × 10^{-4}) after treatment with:	
	10^{-5} μCi of ^{241}Am/ml	5×10^{-5} μCi of ^{241}Am/ml
AAP	6.4 ± 0.2*	16.2 ± 0.9†
AAP + I (0.0183 μm)	9.8 ± 0.4*†	16.7 ± 1.8†
AAP + II (0.0183 to 0.0032 μm)	9.6 ± 2.1*†	12.2 ± 3.4†
AAP + III (0.0032 to 0.0009 μm)	11.8 ± 1.7*†	39.0 ± 2.9
AAP + IV (0.0009 μm)	29.5 ± 3.1	49.2 ± 3.8

[a] $N = 2$, $\alpha = 0.05$, confidence intervals = $\pm 2S_{\bar{x}}$. Means that are not significantly different from one another are denoted by * or †.

TABLE 3. *Effects of four organic fractions on S. obliquus cellular ^{241}Am concentration after 96 h*[a]

Fraction	^{241}Am (μCi/ml)	^{241}Am/cell (pCi × 10^{-6})	
		AAP	AAP† Organics
I (0.0183 μm)	10^{-5}	3.0† ± 0.4 (1.3)[b]	9.8* ± 1.3 (5.2)
	10^{-4}	124.2 ± 9.0 (1.4)	182.0 ± 19.0 (3.1)
II (0.0183 to 0.0032 μm)	10^{-5}	7.7* ± 1.7 (0.9)	3.2† ± 0.7 (2.1)
	10^{-4}	108.7 ± 8.8 (0.4)	26.6 ± 5.8 (2.3)
III (0.0032 to 0.0009 μm)	10^{-5}	8.0 ± 0.7 (1.5)	5.2 ± 1.4 (3.1)
	10^{-4}	98.7 ± 17.4 (1.5)	63.5 ± 3.9 (5.7)
IV (0.0009 μm)	10^{-5}	6.5 ± 1.3 (1.0)	4.1 ± 0.7 (1.9)
	10^{-4}	64.1 ± 7.7 (1.1)	35.1 ± 4.5 (0.9)

[a] $N = 4$, $\alpha = 0.05$, confidence intervals = $\pm 2S_{\bar{x}}$. Means not significantly different from one another are denoted by * or †.

[b] Values in parentheses indicate mean cell density (cells × 10^5/ml) for each treatment combination.

cell densities were approximately equal, indicating that this fraction reduced ^{241}Am uptake due to chelation. Fraction I, however, significantly increased cellular ^{241}Am concentration even though the standing crop of *S. obliquus* cells after 96 h was approximately twice as great, indicating this fraction increases ^{241}Am uptake enough to not only compensate for biological dilution but also significantly increase cellular ^{241}Am concentration.

S. obliquus flocculated fraction I organics after 96 h, causing the organics to be separated from the medium with the algal cells. This flocculation was not observed for fractions II, III, and IV and did not occur in technique blanks containing no algal cells nor in short-term studies. Flocculation of organics cosedimented ^{241}Am, increasing the apparent cellular ^{241}Am concentration. Flocculation of the larger organics by algae may have ecological significance, since ^{241}Am need not be sorbed directly to algal cells to enter the food web. Flocculated organics and algae with associated ^{241}Am may act as a functional particle that is available to grazing zooplankton and fish. Information on the relative availability of ^{241}Am to zooplankton from algae and humics is needed. This mechanism may also serve to remove ^{241}Am from the water column to the sediments.

Concentration factors for ^{241}Am by algae and bacteria decrease with increasing cell density due to biological dilution (15). Consequently, the effect of humic substances on ^{241}Am uptake by *S. obliquus* was confounded by differential growth rates and standing crops, whereas determination of ^{241}Am uptake by *A. hydrophila* was unaffected. Because of biological dilution due to differential growth, reporting ^{241}Am uptake by *S. obliquus* as ^{241}Am activity/cell may be misleading if these effects are not considered. Additional insight into mechanisms of the effects of organics on ^{241}Am uptake may be gained by calculating the following synthetic relationship: % total ^{241}Am removed from medium/cell. This calculated parameter has little ecological significance in itself but reduces the confounding effects of differential grown in the presence of various organic fractions. This calculated parameter is more variable, thus reducing the ability to discriminate between treatment effects.

When represented in this manner, fractions II and III do not show any significant effects on ^{241}Am uptake (Table 4). This does not mean

TABLE 4. *Effects of four organic fractions on percentage of total ^{241}Am removed from the medium per S. obliquus cell after 96 h*[a]

Fraction	^{241}Am (μCi/ml)	% ^{241}Am removed/cell ($\times 10^{-7}$)	
		AAP	AAP + Organics
I (0.0183 μm)	10^{-5}	8.9 ± 2.4†	27.0 ± 7.0*
	10^{-4}	11.3 ± 3.4*†	24.0 ± 17.0†
II (0.0183 to 0.0032 μm)	10^{-5}	70.0 ± 31.6*†	30.1 ± 12.5†
	10^{-4}	106.5 ± 47.6*	52.9 ± 49.8*†
III (0.0032 to 0.0009 μm)	10^{-5}	3.6 ± 1.35*	5.7 ± 2.8*
	10^{-4}	9.0 ± 3.0†	5.8 ± 0.5*†
IV (0.0009 μm)	10^{-5}	13.4 ± 2.8	7.8 ± 2.6
	10^{-4}	6.0 ± 4.0	3.0 ± 1.2

[a] $N = 4$, $\alpha = 0.05$, confidence intervals = $2S_{\bar{x}}$. Means that are not significantly different from one another are denoted by * or †.

that organics in this size range do not bind ^{241}Am but that no significant effects could be determined at natural concentrations. Fraction IV significantly reduced the percentage of total ^{241}Am removed per cell, supporting the contention that this fraction actually reduces ^{241}Am uptake and is not merely an artifact of differential algal population growth. Fraction I, when plotted as percentage of total ^{241}Am removed per cell, caused an increase in ^{241}Am uptake, although this effect was not significant at the higher ^{241}Am medium concentration due to high variability. Increased apparent ^{241}Am uptake, in the presence of fraction I, would be expected due to algal flocculation of this fraction.

Many investigators have studied the effects of humic and fulvic compounds on trace metal uptake by phytoplankton (15, 19, 20, 27, 28, 41, 42). Several investigators have reported increased trace metal uptake by phytoplankton due to metal-organic interactions in natural waters (7, 23, 35), whereas others have reported reduced uptake (14, 18, 30, 33, 34, 41, 44, 52). Shapiro (42) found that higher-molecular-weight fractions of naturally occurring colored organic acids are responsible for most of the chelating capacity in surface waters. Stevenson and Ardakani (47) reported that metals bound to the larger humic fractions were less available to plants. However, in this study the larger organic fractions had no significant effect on ^{241}Am uptake by *S. obliquus* in short-term incubations when present at levels found in nature.

Beside differential growth and flocculation effects, fraction IV reduces ^{241}Am availability during 96-h incubations while increasing ^{241}Am uptake during 4-h incubations. No explanation of these apparently contradictory results are presently available.

As with *S. obliquus*, ^{241}Am uptake by *A. hydrophila* was greater at the higher ^{241}Am medium concentration. Fraction I was the only fraction that significantly affected ^{241}Am uptake by *A. hydrophila* at the lower ^{241}Am concentration, whereas fraction I and IV increased ^{241}Am uptake at the higher ^{241}Am concentration after 4 h (Table 5). There was no flocculation of organics or differential *A. hydrophila* growth during the 4-h study. Increased ^{241}Am uptake due to fraction IV may be due to innate properties of the higher concentration of organics in this fraction (Table 1). Unlike *S. obliquus*, ^{241}Am uptake by *A. hydrophila* was increased by the presence of organics at the higher concentration of ^{241}Am in the medium.

In 24-h incubations, all four organic fractions significantly increased ^{241}Am uptake by *A. hydrophila* (Table 6). Because there was no differential growth among cultures treated with different organic fractions, ^{241}Am per cell accurately represents ^{241}Am uptake by *A. hydrophila* cells. After 24 h of growth, *A. hydrophila* flocculated fraction I organics, some of which were removed from the medium with bacterial cells. Unlike *S. obliquus* cultures where organics reduced ^{241}Am availability, the flocculation and sedimentation of fraction I organics by *A. hydrophila* did not reverse the apparent availability trend but enhanced it.

ACKNOWLEDGMENTS

This research was supported by contract E-38-819 between the University of Georgia and the U.S. Energy Research and Development Administration.

Aid in sample preparation and analysis by L. A. Briese and R. R. Geiger is appreciated. J. W. Bowling, C. B.

TABLE 5. *Effect of four organic fractions on A. hydrophilia cellular ^{241}Am concentration after 4 h*[a]

Fraction	^{241}Am/cell (pCi × 10^{-6}) after treatment with:	
	10^{-5} μCi of ^{241}Am/ml	5×10^{-5} μCi of ^{241}A m/ml
0.5% TSB	4.2 ± 0.9*	4.7 ± 1.2*
0.5% TSB ± I (0.0183 μm)	6.2 ± 1.9†	6.0 ± 0.9†
0.5% TSB + II (0.0183 to 0.0032 μm)	4.1 ± 0.9*	4.3 ± 1.3*
0.5% TSB + III (0.0032 to 0.0009 μm)	3.0 ± 0.6*	4.2 ± 0.4*
0.5% TSB + IV (0.0009 μm)	3.5 ± 0.2*	9.6 ± 1.0

[a] $N = 2$, $\alpha = 0.05$, confidence intervals = $\pm 2S_{\bar{x}}$. Means that are not significantly different from one another are denoted by * or †.

TABLE 6. *Effect of four organic fractions on A. hydrophila cellular ^{241}Am concentration after 96 h*[a]

Fraction	^{241}Am (μCi/ml)	^{241}Am/cell (pCi × 10^{-9})	
		0.5% TBS	0.5% TSB + Organics
I (0.0183 μm)	10^{-5}	7.2 ± 0.3	15.7 ± 1.1
	10^{-4}	46.2 ± 10.6	141.0 ± 27
II (0.0183 to 0.0032 μm)	10^{-5}	5.7 ± 1.2*	2.1 ± 0.3*
	10^{-4}	28.4 ± 4.2	19.5 ± 2.1
III (0.0032 to 0.0009 μm)	10^{-5}	5.4 ± 1.3	6.3 ± 1.6
	10^{-4}	113.1 ± 16.3	196.9 ± 24.0
IV (0.0009 μm)	10^{-5}	6.8 ± 1.9	11.9 ± 1.3
	10^{-4}	59.0 ± 6.7	137.1 ± 13.0

[a] $N = 4$, $\alpha = 0.05$, confidence intervals = $\pm 2S_{\bar{x}}$. Means that are not significantly difference from one another are denoted by *.

Fliermans, R. W. Gorden, and T. C. Hazen kindly commented on the manuscript.

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