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# Accumulation of Am-241 and Cm-244 from Water and Sediments by *Hyalella* sp. and *Tubifex* spp.

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Transuranic elements (atomic elements > 92) have been introduced into the environment since the 1940's as a result of atmospheric testing of nuclear weapons, discharge of low-level nuclear wastes, and nuclear fuel reprocessing. The reported toxicity of these elements (Edsall 1976) has generated considerable public health concern, although the environmental concentrations are very low. Many of these isotopes are long-lived, but it is difficult to predict their long-term patterns of biogeochemical cycling because they have been introduced into the environment so recently and have no natural analogs. Consequently, considerable research effort has been directed toward the determination of processes that affect the transport and fate of transuranic elements in natural environments (Hanson 1980, IAEA 1981, Pinder et al 1987). These efforts have emphasized terrestrial environments because they present the most direct pathway to man, especially through food crops, and the element plutonium because it was the most abundant transuranic element in atmospheric fallout. Relatively little attention has been given to freshwater environments (Thiels 1982) or to other transuranic elements (Watters et al. 1980). This paper reports on the bioaccumulation of americium and curium by freshwater invertebrates in laboratory experiments. Uptake by benthic invertebrates will affect both the biogeochemical cycling of these elements and the potential exposure of man through accumulation in aquatic food chains.

Am and Cm isotopes are produced by nuclear reactions in commercial reactors and are major components of high level wastes. Also, the global inventory of Am-241 is increasing because it is a decay product of plutonium-241. In aquatic environments both Am and Cm occur in the trivalent oxidation state and may be expected to behave similarly, although Beasley and Cross (1980) cautioned against direct extrapolation from one element to the other. Both elements bind strongly to sediments which may be the principal source for uptake by benthic organisms in freshwater and marine environments.

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The principal objectives of this research were: 1) To determine the extent of bioaccumulation of Am and Cm for freshwater species, 2) To compare bioaccumulation from water with bioaccumulation from various types of sediment particles, and 3) To evaluate the similarities and differences in the behavior of Am and Cm.

## MATERIALS AND METHODS

An amphipod (Hyalella sp.) and oligochaetes (Tubifex spp.) were chosen as the test organisms because they are: 1) common taxonomic groups of freshwater benthic communities, 2) important prey items for fish and 3) easily maintained in the laboratory. Hyalella were obtained from Dr. Freida Taub (School of Fisheries, University of Washington) and Tubifex were bought from a commercial source in Seattle, Washington. Prior to use in experiments, all organisms were rinsed with distilled, deionized water to remove any debris that was attached to their external surfaces and held in filtered Lake Washington water for less than five hours.

Organisms were exposed to radioisotopes that were dissolved in filtered lake water or adsorbed on particulate surfaces. Specific activity of the $^{241}Am$ source solution was $2.3 \times 10^5$ dpm $mL^{-1}$ (3,800 Bq $mL^{-1}$) and the $^{244}Cm$ source was $6.5 \times 10^6$ dpm $mL^{-1}$ (10,800 Bq $mL^{-1}$). Each isotope was stored in 0.5N $HNO_3$. To provide labeled lake water, 600 uL of Am source or 60 uL of Cm spike were added to 100 mL of filtered (0.45 um) Lake Washington water. The pH was adjusted to 7.4 by dropwise addition of 0.5N NaOH and buffered with $1.7 \times 10^{-4}$ M $KH_2PO_4$ and $6.1 \times 10^{-4}$ M $Na_2HPO_4$. The solution was then allowed to equilibrate overnight before being used in experiments.

Labeled particulates were: 1) <0.044 mm particles from a National Bureau of Standards (NBS) river sediment (Standard reference material 4350), 2) <0.158 mm particles of quartz sand, and 3) <0.104 mm detrital particles from an aquatic macrophyte. To produce organic detritus, Myriophyllum spicatum (Eurasian milfoil) was collected from Lake Washington, oven dried at 70°C, ground with a porcelain mortar and pestle and passed through a 0.104 mm sieve. Quartz sand was washed with 1.0 N $HNO_3$ to remove coatings of metal oxides and then rinsed in distilled water. To label particles 2.1 gm of detritus and 21 gm of sand or NBS sediment were suspended in 100 ml of distilled deionized water; 600 ul of $^{241}Am$ or 60 ul of $^{244}Cm$ spike was added and the pH was adjusted to approximately 7.5 with 0.5N NaOH. The suspension was stirred for 24 hours and centrifuged to remove particles from suspension. Particles were then dried at 47°C.

Organisms were exposed to one of the isotopes from one of four sources (water, sand, detritus or NBS sediments). Exposure occurred in a 15-ml glass test tube with 10 ml of filtered, buffered Lake Washington water. For particulate experiments, either 0.07 (detritus) or 0.7 gm (sand, NBS sediment) of labeled substrate was added to each tube. Five organisms were added to each source-isotope combination. Triplicate experiments were

sampled after 1 hour, 1 day and 5 days of continual exposure.

Individual test tubes were sampled only once at which time 5 ml of water was pipetted off and filtered through a tared 0.45 um filter. Four ml of filtrate were collected to determine dissolved activity of the isotope. The filter was dried at 47°C, weighed and analyzed for activity of suspended particles (e.g. radiocolloids, microorganisms, fecal particles). Invertebrates were removed with stainless steel instruments; rinsed in pH adjusted, distilled-deionized water; placed in tared weighing dishes and dried at 47°C for 24 hours. Filters and organisms were placed in glass test tubes with 1 ml of 12M $H_2SO_4$ for 24 hours and then diluted with 10 ml of distilled-deionized water. Two ml of this solution was filtered through a 0.45um Millipore filter and 1 ml of the filtrate was collected to determine the activity on the filters or in the organisms. After the organisms were removed, particulates were placed in tared weighing dishes, dried at 47°C for 24 hours and treated with 12M $H_2SO_4$ to desorb the radioisotopes, diluted with distilled-deionized water and filtered through 0.45um filters to obtain a sample for counting. Neither the organisms nor the particulates were completely dissolved by the acid, but the isotopes were completely solubilized (Stohr 1983).

Both isotopes emit alpha particles with energies between 5.4 and 6.0 MeV; a single alpha is emitted for each disintegration. Activity was measured in a Packard Tri-Carb Model 1330 liquid scintillation counter using methods described by Wurtz et al. (1986). Specific activity was calculated by dividing sample activity, following background correction, by sample weight. Triplicate values were averaged for each experimental component and results are presented as mean values $\pm$ one standard deviation. Counting errors were negligible relative to the variation among experiments.

## RESULTS AND DISCUSSION

Measurable concentrations of $^{241}Am$ were transferred to Hyalella from labelled water, sand and detritus (Fig. 1). Accumulation from water occurred rapidly to produce concentrations in the organisms after 1 hour that were nearly 2 orders of magnitude higher than the soluble concentrations. An additional increase was observed during the first day but no significant changes occurred after day 1. Uptake of $^{241}Am$ from labelled particulates occurred more slowly and to a lesser extent than accumulation from water. After 1 hour organisms in the detritus and sand experiments had measurable amounts of $^{241}Am$, although the average concentration in organisms increased throughout the experiment for both particulates. In the experiments with NBS standard sediment there was no measurable uptake by Hyalella. The differences in accumulation by organisms correspond well to differences in the concentration of soluble $^{241}Am$ (Fig.1). This suggests that soluble Am provides the principal route of uptake and radionuclides must first be desorbed from sediments before they are available for bioaccumulation.

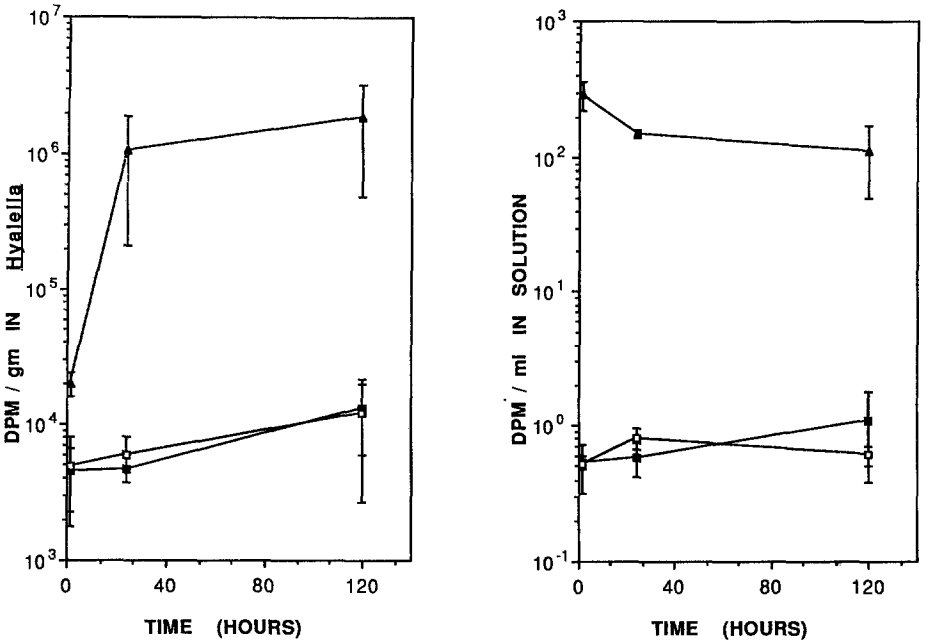


Figure 1. Activity of $^{241}Am$ in Hyalella and in solution during a five-day uptake experiment. Plotted values are means (n=3) ± 1 SD. ▲ = water, □ = sand, ■ = detritus.

Accumulation of $^{241}Am$ by Tubifex is very similar to accumulation by Hyalella (Fig. 2). Again, there was measurable uptake from labelled water, sand and detritus after 1 hour; the concentration of $^{241}Am$ in Tubifex is greatest in experiments with labelled water; uptake from detritus and sand increased throughout the experiments, and there was no measurable release of $^{241}Am$ from the NBS sediment. After 5 days the activity of $^{241}Am$ accumulated from the different sources is nearly equivalent for Tubifex and Hyalella.

The soluble concentration of $^{241}Am$ is greater in the Tubifex experiments than in the Hyalella experiments. This suggests that Tubifex may excrete metabolites that form soluble complexes with $^{241}Am$ and promote desorption but are not accumulated by organisms. An effect of metabolites is also suggested by comparing the soluble concentration in controls (no organisms) to the uptake experiments with labelled water. In the controls, the soluble concentration of $^{241}Am$ is initially greater than in the experiments with organisms but decreased throughout the experiment due to adsorption on the test tube walls. After 5 days the soluble concentration is higher in containers with organisms, presumably because metabolic products form soluble complexes with $^{241}Am$. It has been shown previously that organic ligands alter adsorption behavior of $^{241}Am$ to freshwater (Clayton et al. 1982) and estuarine sediments (Sibley and Clayton 1985). Wurtz et al. (1986) argued that exometabolites from bacteria can alter the adsorption behavior of $^{241}Am$. Exometabolites that complex metals are well

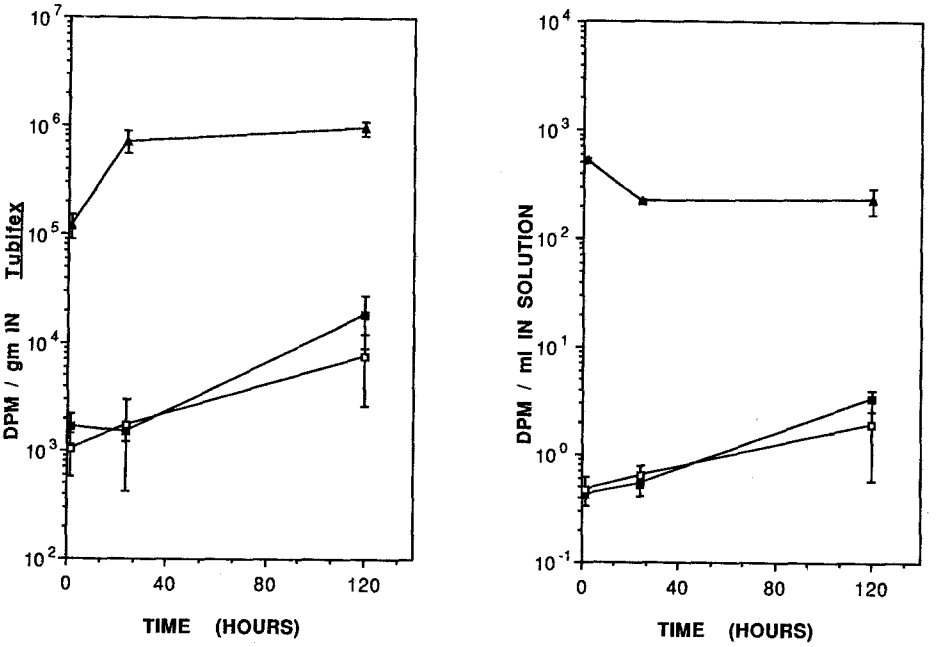


Figure 2. Activity of $^{241}Am$ in Tubifex and in solution during a five-day uptake experiment. Plotted values are means (n=3) ± 1 SD. ▲ = water, □ = sand, ■ = detritus.

known for phytoplankton and bacteria, although there is little information on exometabolites of invertebrates. Fish and Morel (1983) reported that the crustacean species Daphnia magna excretes organic compounds that form moderately strong complexes with copper. It seems likely that similar ligands are excreted by Tubifex and form complexes with Am.

Curium-244 behaved somewhat differently than $^{241}Am$ in our experimental system. Figure 3 plots the activity of $^{244}Cm$ in Hyalella and Tubifex and the activity in solution as a function of time for the experiments with labeled water. Like $^{241}Am$ the soluble activity in the controls decreases throughout the experiment as Cm adsorbs to the glassware. Soluble activity of Cm increases with time in both Hyalella and Tubifex experiments and exceeds the activity in the controls by nearly an order of magnitude after 5 days. It appears, therefore, that metabolites have a greater effect on Cm than on Am.

Accumulation of $^{244}Cm$ from labelled water by Hyalella and Tubifex is very rapid (Fig. 3). After 1 hour approximately 15% of the added Cm has accumulated in Tubifex and 35% has accumulated in Hyalella. After 5 days 75-85% of $^{244}Cm$ is found in the organisms compared to approximately 35% of $^{241}Am$. Uptake of $^{244}Cm$ by Hyalella is faster than the uptake by Tubifex and the specific activity is greater in Hyalelle throughout the experiment. This is in contrast to the results for $^{241}Am$ which produced comparable specific activity in the two species after 5 days.

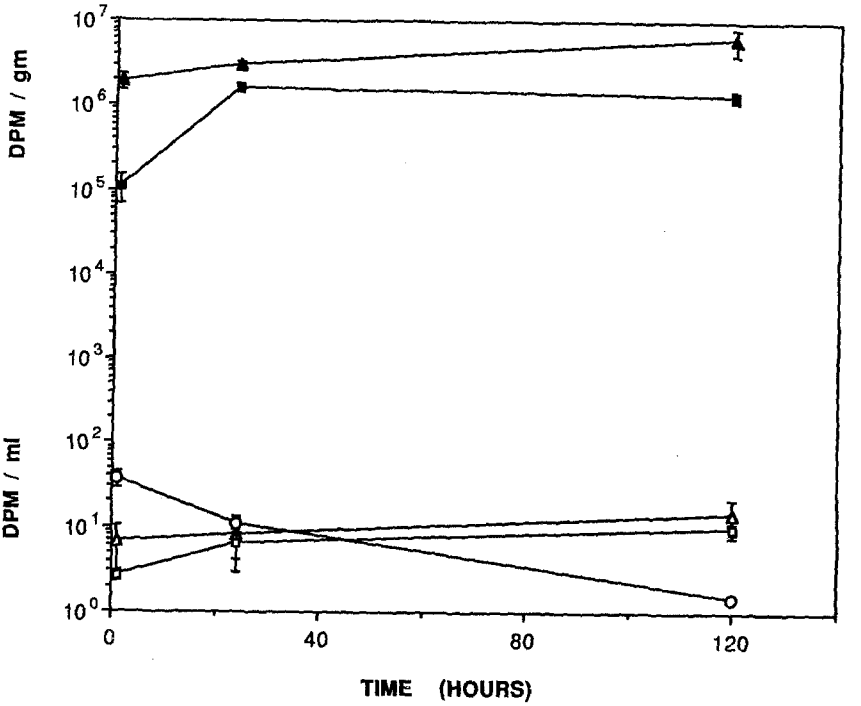


Figure 3. Activity of $^{244}Cm$ in organisms and in solution during five-day uptake experiment. Plotted values are means (n=3) ± 1 S.D. ▲ = Hyalella, ■ = Tubifex, ○ = Control. Control is labeled solution with no organisms.

Soluble activity of $^{244}Cm$ is lower (Fig. 3) than the activity of $^{241}Am$ (Figs.1 and 2) but the activity in the organisms is higher. Thus, Cm has a higher affinity than Am for the organisms and has higher concentration factors (Sibley and Stohr 1986). Pentreath et al (1986) reported that concentration factors are higher for Cm than for Am in the marine benthic alga, Ascophyllum nodosum.

In the experiments with labelled particulates, we never obtained measurable concentrations in organisms. This is a surprising observation because the activity of soluble $^{244}Cm$ is consistently greater than the activity of soluble $^{241}Am$ in these experiments (Stohr 1983). Extrapolating from the results in labelled water, we would expect much higher activity of $^{244}Cm$ in the organisms. Thus, the relative behavior of Am to Cm appears to be different for experiments with labelled particulates than for labelled water. The most likely explanation for these differences in behavior is that particulates alter the physico-chemical speciation of Am and Cm and the subsequent bioaccumulation of these isotopes. For example, Sanchez et al. (1985) have recently shown that the presence of particulates can significantly alter the oxidation-reduction chemistry of plutonium. If similar effects occur for Am and Cm it could contribute to the results we obtain. We cannot determine if the differences between $^{241}Am$ and $^{244}Cm$ occur in the speciation of soluble forms or at the bioaccumulation stage.

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