IN: Transuranics in Natural Environment, M. G. White and P. B. Dunaway (eds.). NVO-178, Las Vegas, Nevada. p. 531-543. 1977.

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EFFECT OF NATURALLY OCCURRING ORGANICS ON PLUTONIUM-237 UPTAKE BY ALGAE AND BACTERIA

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ABSTRACT

Naturally occurring organics were concentrated from Skinface Pond, near Aiken, South Carolina and separated into four nominal diameter size fractions (F I > 0.0183; 0.0183 > F II > 0.0032; 0.0032 > F III > 0.009; F IV < 0.009 μm) by membrane ultrafiltration. Each fraction was introduced into Scenedesmus obliquus and Aeromonas hydrophila cultures at concentrations equal to those found in nature to determine their effects on ^{237}Pu uptake. Plutonium-237 uptake was determined in log phase cultures after 6 hr incubations. The initial plutonium concentration in each flask was 1.1×10^{-4} μCi/ml $^{237}Pu^{+4}(NO_3)_4$. Fractions I and II significantly reduced ^{237}Pu uptake by S. obliquus, while F IV increased uptake and F III had no effect. Plutonium-237 uptake by A. hydrophila was not significantly different in the presence of F I, F II or F III than tryptic broth medium alone, while F IV significantly increased ^{237}Pu uptake.

INTRODUCTION

While plutonium presently exists at very low concentrations in the biosphere, trophic biomagnification and possible localized contaminations may result in increased plutonium concentrations in organisms of higher trophic levels. Algae and bacteria form the base of aquatic food webs and concentrate plutonium greatly over water concentrations (Noshkin, 1972; Folsom et al., 1975; Gromov

and Spitsyn, 1974a, 1974b; Giesy and Paine, 1977a). Cycling processes and biological uptake of Pu must be understood prior to environmental releases so that rational assessments of its hazards can be determined. Accumulation of plutonium by algae and bacteria is dependent upon the aqueous forms of plutonium (Andelman and Rozzell, 1970; Noshkin, 1972). While much is known about plutonium separations chemistry, little is known about the environmental chemistry and speciation of plutonium (Noshkin, 1972).

Francis (1973) stated that chelation with naturally occurring organic soil components may be important in the movement of transuranics in natural food chains leading to man. Szalay (1964) suggested that humic acids be used in the disposal of radioactive wastes because these organic compounds immobilize radionuclides. Pillai and Mathew (1976) studied the effects of humics on Pu solubilization in sea water but emphasized that further information is needed on the chemical behavior and influence of humics on Pu behavior in water. Routson et al. (1976) reported that Pu^{+4} tends to form complexes with many organic ligans such as those in soil. While Pu-humic interactions have been investigated (Bondietti, et al., 1975; Routson, et al., 1976), no studies have considered the effect of humics and fulvics on the availability of plutonium to aquatic biological systems.

Recalcitrant, naturally occurring organic compounds, variously 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 the chemical processes of nearly all surface waters (Schnitzer and Khan, 1972). Organic ligans, which can form complexes, with metals are important in determining the form, movement, and availability of trace metals in natural waters (Rashid and Leonard, 1973; Andelman, 1974; Pittwell, 1974; Jackson, 1975; Giesy, 1976). The distribution of organic carbon between various nominal diameter fractions varies spatially and temperally. Because of this variability in nominal diameter of organics an understanding of the effects of the various sized fractions must be obtained instead of total organic ligand.

The surface waters of the Southeast 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 Pu cycling and fluxing processes in aquatic ecosystems. The objective of this study was to determine the effects of various sized organics on Pu uptake by an algal and bacterial species.

MATERIALS AND METHODS

Axenic cultures of Scenedesmus obliquus (Türp) Kütz (strain #1592) were obtained from the Indiana University Culture Collection. Aeromonas hydrophila (strain #7966) was obtained from the American Type Culture Collection. Stock, axemic S. obliquus cultures were maintained in 200 ml Algal Assay Procedure (AAP) medium aerated with sterile air (Anon, 1971). Stock algal cultures were checked

periodically for bacteria contamination by plating on peptose agar, incubating in tryptic soy broth (TSB) and microscopic examination. Stock and experimental cultures were incubated at 24 ± 2°C under 4035 lux illumination from balanced spectrum "Growlux" fluorescent bulbs on a 16 hr light-8 hr dark regime. Algal inocula were taken from 10 day old S. obliquus cultures with cell densities of 4.2×10^5 cells $\cdot$ ml^{-1} and relative growth rate (K' 0.68 day^{-1}). Initial S. obliquus cell densities were concentrated by centrifugation to between 1 and 2×10^5 cells $\cdot$ ml^{-1} in experimental flasks. Stock A. hydrophila cultures were maintained in AAP-tryptic soy broth (TSB) medium (Difco). A. hydrophila inocula were drawn from 24 hr cultures with cell density of 4.5×10^6 cells $\cdot$ ml^{-1} (K' = 3.6 day^{-1}) to make an initial cell density of 5×10^5 cells $\cdot$ ml^{-1} in experimental flasks. S. obliquus and A. hydrophila were incubated with $^{237}Pu^{+4}$ for 6 hr on a rotary shaker at 200 rpm. Algal and bacterial cell densities were determined, using calibrated phytoplankton (0.1 ml) and Petroff-Hausser counting chambers respectively.

Plutonium-237 was obtained from Oak Ridge National Laboratory and assayed by the Savannah River Laboratory. Plutonium was prepared as $^{237}Pu^{+4}$ (Table 1). Plutonium stocks (1.1×10^{-2} µCi $\cdot$ ml)were stored in 0.5 N HNO_3 in polyethylene.

Water samples were collected from Skinface Pond, Aiken, Co., South Carolina. Particulates of nominal diameter ≥ 0.15 µm were removed and the remaining dissolved and colloidal constituents fractionated and concentrated by membrane ultrafiltration (Giesy and Briese, 1977; Giesy and Paine, 1977b).

Uptake experiments were conducted in 100 ml medium in 300 ml Erlenmeyer flasks. One milliliter of $^{237}Pu^{+4}$ stock was added to AAP or AAP + 0.5% TBS (bacterial uptake) and adjusted to pH 4.5 with 1.0 N NaOH using a microburette. The volume of NaOH required to adjust the experimental solutions containing organics and $^{237}Pu^{+4}$ spike had been previously determined. Organics were present in experimental media at the concentration at which they were found in nature. The resulting experimental media contained 1.1×10^{-4} µCi $^{237}Pu^{+4}$ at pH 4.5. Solutions were mixed and allowed to stand 5 min before algal or bacterial inoculations.

Algae and bacteria were separated from experimental media by centrifugation into a phthalate separator (Giesy and Paine, 1977b). 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 or Pu polymerization. Reagent blanks, controls and experimentals were analyzed for ^{237}Pu using a GeLi detector interfaced to an INOTEC 4000 channel pulse height analyzer.

The experimental design was a randomized block design. Since all replicates could not be centrifuged concurrently, experimental units were blocked orthogonally over time. Significance of treatment effects were tested using standard analysis of variance techniques and means separated with Student-Newman Keubs multiple range test. Significance was tested for at the $\alpha = 0.05$ level.

Table I. Flow chart of $^{237}Pu^{+4}$ preparation procedure.

I. REMOVAL OF ORGANIC MATTER

A. Place Pu solution in HNO_3 solution in beaker.
B. Evaporate to dryness. Do not bake residue.
C. Dissolve residue in 2 ml con. HNO_3. Evaporate to dryness. Do not bake residue.
D. Repeat Step I C.
E. Add 2 ml 30% H_2O_2 to residue. Evaporate to dryness. Do not bake residue.
F. Repeat Step I E.
G. Add 2 ml con. HNO_3. Evaporate to dryness. Do not bake residue.
H. Repeat Step I G.

II. ADJUST ALL Pu TO Pu^{+4} OXIDATION STATE

A. Add 10 ml 1N HNO_3 to residue.
B. Add 1 ml 1M $NaNO_3$. Heat 30 min. (light boil, 400°C) for 30 min. with stirring. Cool.

III. OXIDIZE Pu^{+4} TO Pu^{+5}

A. Add 0.5 M $KMnO_4$ dropwise with stirring until pink color persists. Add 0.2 ml 0.5 M $KMnO_4$.
B. Heat for 30 min. (250°C). Cool to 25°C.

IV. REDUCE Pu^{+5} TO Pu^{+3}

A. Add 1 M $Fe(NH_2SO_3)_2$ dropwise with stirring until solution is clear. Add 0.25 ml 1 M $Fe(NH_2SO_3)_2$.
B. Stir 30 min. Do not heat.

V. OXIDIZE Pu^{+3} TO Pu^{+4}

A. Add 4 ml 1 M $NaNO_2$.
B. Heat for 30 min.
C. Wash into 25 ml 8 N HNO_3.

VI. ADSORB Pu^{+4} ONTO ANION EXCHANGE COLUMN

A. Add 8 ml (HNO_3) to solution
B. Pass solution through column (5 ml volumes) Dowex(R) - 1 x 4, 20-50 mesh resin.
C. Collect effluent. Wash beaker with 2 ml 8 N HNO_3. Pass washes through column.
D. Repeat VI C.

VII. ELUTE Pu^{+4} FROM ANION EXCHANGE COLUMN

A. Wash column with 100 ml 0.5 N HNO_3. Collect effluent as Pu^{+4}.

RESULTS

Total organic carbon (TOC) in Skinface Pond was 31.1 mg/l (Table 2), which is approximately four times the world average for rivers (Garrels and MacKenzie, 1971) which is typical of southeastern aquatic systems (Beck et al., 1974). More than 80% of the TOC was comprised of F III and F IV (Table 2). Fraction I and F II both significantly reduced ^{237}Pu uptake by S. obliquus (Table 3). Fraction III, which contained 36% of the TOC had no significant effect on ^{237}Pu uptake by S. obliquus while F IV significantly increased ^{237}Pu uptake.

A similar trend was observed for ^{237}Pu uptake by A. hydrophila (Table 4). Both F I and F II reduced ^{237}Pu uptake below that in TSB medium alone. As with S. obliquus F III did not significantly affect ^{237}Pu uptake by A. hydrophila, while F IV significantly increased ^{237}Pu uptake.

No polymerization was observed in these experiments. Less than 5% of the plutonium was lost by plating to glass flasks with plating losses less in flasks containing organics.

DISCUSSION

Uptake is herein defined as the association of ^{237}Pu with algal or bacterial cells. Uptake does not imply an active mechanism and no attempt was made to determine if ^{237}Pu was associated with the surface or interior of cells.

Oxidation state may be important in determining the physical and chemical reactions which Pu will undergo in the environment (Rouston et al., 1976). Wahlgren et al. (1976) reported Pu^{+4} to be the predominant oxidation state in Lake Michigan water. For this reason a rigorous procedure was used to assure that all of the ^{237}Pu was in the IV oxidation state at the beginning of the uptake studies. The environmental chemistry of Pu is also pH dependant (Rouston et al., 1976), as is the complexation chemistry of humic acids (Stevenson and Ardakani, 1972). For this reason the pH of experimental media was adjusted to 4.5, which is typical of many southeastern surface waters such as Skinface Pond. While Pu^{+4} self polymerization has been observed, this phenomenon was probably not a major Pu transformation in this study. Polymerization is directly proportional to Pu concentration and inversely proportional to pH (Rouston et al., 1976).

Many investigators have studied the effects of humic and fulvic compounds on trace metal uptake by phytoplankton (Goldberg, 1952; Shapiro, 1957; Giesy, 1976). Several investigators have reported increased trace metal uptake by phytoplankton due to metal organic interactions (Burk, et al., 1932; Provasoli and Pinter, 1959; Johnson, 1964), while others have reported reduced uptake due to humic acids (Provasoli et al., 1957; Provasoli, 1963; Prakash and Rashid, 1968; Siegal, 1971; Giesy, 1976). Shapiro (1966) found that higher

Table 2. Organic carbon distribution in ultrafilter fractionation ranges of Skinface Pond water.

Ultra-filter	Fraction	Nominal Molecular Weight	Organic carbon (mg/l)	Organic carbon (%)
XM-300	FI* > 0.0183μm	FI > 300,000	2.2	7
PM-10	0.0183 > FII > 0.0032μm	300,000 > FII > 10,000	3.8	12
UM-05	0.0032 > FIII > 0.0009μm	10,000 > FIII > 500	11.1	36
UM-05	FIV > 0.0009μm	FIV > 500	14.0	45
TOTAL			31.1	100

*f = Organic fraction isolated

Table 3. Effects of four organic fractions on S. obliquus cellular ^{237}Pu concentration after 6 hr.

Treatment	Final cell density (cell x $10^5 \cdot ml^{-1}$)	$^{237}Pu^{+4}$ Removed From Medium	
		Removed/Cell (10^{-5} pCi $^{237}Pu \cdot cell^{-1}$)	% Total Removed
AAP	$1.1 \pm 0.18^{A*}$	32 ± 6^{B}	29
AAP + F I	1.6 ± 0.36^{A}	6 ± 2^{C}	9
AAP + F II	1.3 ± 0.22^{A}	12 ± 4^{C}	14
AAP + F III	1.3 ± 0.30^{A}	29 ± 6^{B}	34
AAP + F IV	1.3 ± 0.28^{A}	52 ± 7	62

*N = 4, $\alpha = 0.05$, confidence intervals = $\pm 2 S_{\bar{x}}$. Means which are not significantly different from one another are denoted by A, B or C.

Table 4. Effects of 4 organic fractions on A. hydrophila cellular ^{237}Pu concentration after 6 hr.

Treatment	Final cell density (cell x $10^7 \cdot ml^{-1}$	$^{237}Pu^{+4}$ Removed From Medium	
		Removed/Cell 10^{-8} pCi $^{237}Pu^{+4} \cdot cell^{-1}$	% Total Removed
TSB	$4.3 \pm 2^{A*}$	14 ± 6^{C}	5.4
TSB + F I	5.1 ± 2.2^{A}	7 ± 2^{B}	3.2
TSB + F II	3.6 ± 1.4^{A}	10 ± 2^{B}	3.2
TSB + F III	3.1 ± 1.2^{A}	15 ± 2^{C}	4.2
TSB + F IV	5.6 ± 2.1^{A}	51 ± 9	26.0

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

molecular weight fractions of naturally occurring colored organic acids are responsible for most of the chelating capacity in surface waters. Stevenson and Ardakani (1972) reported that metals bound to the larger fractions were less available to plants and less mobile in soil solution. In this study the larger diameter organics reduced ^{237}Pu uptake, presumably by chelating an otherwise sequestering Pu. The smaller diameter organics greatly increased Pu uptake. This may be due to decreased Pu precipitation and adsorption to glass. Organic matter in soil solutions decreased Pu availability to plants (Schulz et al., 1976). Pillai et al. (1976) found humics maintained more Pu in solution than sea water alone but precipitated removing Pu over a long period of time. Smaller diameter organics may also facilitate Pu uptake by crossing the cell membrane as a Pu-organic complex or by juxtapositioning Pu on the cell. Metal organic complexes may readily penetrate cell membranes, thus chelating agents may transport metals into cells in quantities greater than would normally occur (Doyle et al., 1974).

Low molecular weight humic acid fractions and fulvic acids may penetrate the cell membranes of phytoplankton (Prakash et al., 1973) but the higher molecular weight humic acids (F I and F II) probably do not. Prát et al. (1961) and Prát and Pospisil (1959) report that humic acids cannot penetrate plant cell membranes. Humic substances that penetrate cell membranes are inhibitory to all cellular processes (Prát, 1968). The important point is that all naturally occurring organics do not increase or decrease Pu uptake but some increase Pu uptake while others cause a decrease. Waris (1953) suggested that humic substances may directly affect the cytoplasmic membrane. Chaminade (1956) reported humic acids stimulate growth of violet epidermal cells by allowing mineral transport across the cytoplasmic membrane, while Saunders (1957) stated that humic acids may stimulate cell membranes of phytoplankton, thereby allowing an influx of bound metals.

ACKNOWLEDGMENTS

This research was supported by Contract E(38-1)-819 between the University of Georgia and the U. S. ERDA. R. M. Wallace, and E. L. Wilhite helped with Pu^{+4} preparation. R. Geiger and L. A. Briese aided in data collection and J. C. Corey helped with all phases of the study.

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