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PLUTONIUM UPTAKE BY THE GREEN ALGA *Scenedesmus Obliquus* (Turp) Kutz, AS A FUNCTION OF ISOTOPE AND OXIDATION STATE

by

M. F. Tkacik

Savannah River Laboratory
E. I. du Pont de Nemours & Co.
Aiken, South Carolina 29801

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by

Michael Francis Tkacik

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Savannah River Laboratory
E. I. du Pont de Nemours & Co.

Savannah River Ecology Laboratory
University of Georgia

School of Public Health
University of South Carolina

Dean of the Graduate School

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ABSTRACT

Uptake of four plutonium forms by the green alga *Scenedesmus obliquus* (Türp) Kütz was studied. Plutonium accumulated by this alga was linearly related to plutonium concentration. There was no significant differences ($p = 0.05$) in algal accumulation of ^{238}Pu or ^{239}Pu in the Pu^{+4} or Pu^{+6} oxidation state at the concentrations studied. Liquid scintillation counters interfaced to multichannel analyzers were suitable for determining the plutonium content of the samples and were accurate over a wide range of sample activities. Plutonium-237 was an effective tracer for plutonium chemical recovery.

INTRODUCTION

Since its discovery in 1941 (Seaborg *et al.*, 1946), plutonium has become one of the most studied elements in the periodic table. However, for a number of reasons the extent of experimental aquatic data available in the literature is quite limited (Paine, 1974). Originally plutonium was considered as relatively unimportant as an environmental hazard because of its low solubility and absence of penetrating gamma radiation. As a result, other radionuclides, such as strontium-90 and cesium-137, received more attention from radioecologists because these were readily transported through the food web (Bair, 1974). However, plutonium is primarily an alpha emitter and is hazardous when deposited inside the body (Zolotar, 1975). For this reason, Tamplin and Gofman (1970) claim that plutonium introduces risks, such as cancer induction, to those exposed to it.

A large number of radioelements exist which are toxic, not because of their chemical properties, but because of the ionizing radiation they emit. Plutonium, as a radiation hazard, is, therefore, not unique; indeed, other radioelements are known to exist which have comparable or greater toxicity per unit mass. However, plutonium-239 in particular, justifies special attention

because of its long half-life and the large quantities of this isotope that are expected to accumulate with increased nuclear power facilities (Medical Research Council, 1975). Knowledge and understanding of plutonium's complex cycling is necessary to predict the effects of possible inadvertant releases (Bennett, 1974).

Phytoplankton can be one of the first trophic levels in which soluble compounds are involved, and algal species can quickly accumulate various trace elements including radionuclides deposited in aquatic systems (Gromov and Spitsyn, 1974a). *Scenedesmus obliquus* is a commonly occurring species in many fresh water systems, and is, therefore, of ecological importance.

The objectives of the present study were three-fold: 1) to investigate the effect of chemical valence state and isotope on the quantity of plutonium associated with algal cells; 2) to develop a rapid and inexpensive method of plutonium determination in aquatic systems; and 3) to investigate the use of plutonium-237 as a tracer for chemical yield determinations during sample preparation and analysis.

PLUTONIUM IN AQUATIC ECOSYSTEMS

A Review of the Literature

Plutonium has entered ecosystems by four mechanisms:

1) close-in fallout from nuclear explosive testing;
2) world-wide fallout from nuclear detonations; 3) atmospheric burn-up of NASA's System for Nuclear Auxiliary Power (SNAP) systems; and 4) fluid wastes from chemical processing and reactor operations (Volchok, 1974).

Plutonium is widely distributed in the oceans as a result of these activities (Bowen, 1975), and ^{238}Pu contributes more to the radiation exposure of many marine species than either ^{90}Sr or ^{137}Cs (Noshkin, 1972).

Preliminary analytical data on marine organisms found $^{239,240}\text{Pu}$ (^{239}Pu and ^{240}Pu are virtually indistinguishable because of similar alpha energies; $^{239,240}\text{Pu}$ is referred to throughout as ^{239}Pu) to be highly concentrated in fish, shellfish, plankton and algae (Pillai *et al.*, 1964). Although recent investigators have developed more advanced radiochemical techniques (Wong, 1971; Hodge *et al.*, 1974), Pillai's study directed attention to the presence and distribution of plutonium in the environment.

Plutonium in the North Sea was assessed by Murray and Kautsky (1975). The plutonium distribution in this area is thought to be influenced by the discharge of activity from three nuclear reprocessing plants, La Hague in Northern France, Windscale in Northwest England, and Dounreay in Northern Scotland as well as fallout from atmospheric testing. Plutonium concentrations in sea water samples from the English Channel ranged from 10.6 to 0.0004 pCi/Kg, the highest level was 15 times greater than fallout levels, strongly indicating the effects of waste discharges at La Hague (Murray and Kautsky, 1975).

British Nuclear Fuels, Ltd., discharges plutonium at the extraordinary rate of 104 Ci per month. Hetherington *et al.* (1975) surveyed its discharge region for plutonium pathways in foodstuffs. Their results gave plutonium concentrations of sea water to be 0.002 pCi/g, fish flesh 0.0004, *Porphyra* seaweed 1.0, sediment 10.0, and *Mytilus edulis* visceral mass had plutonium concentrations of 16 pCi/gram. According to Fisheries Radiological Laboratory (Br.) the discharges are well within the permissible limits authorized (Hetherington *et al.*, 1975). Frazier and Guary (1975) reported plutonium concentrations in marine organisms off the coast of France. Uptake levels of lichen (0.171 pCi/g), mollusks (.0082 pCi/g), and fish (.00004 pCi/g) led

them to the conclusion that there is an accumulation gradient related to phylogenetic position.

Biological concentration of plutonium by marine species could be of importance in moving plutonium (Bowen *et al.*, 1971), particularly to waters of the open ocean. A mean concentration factor of 2×10^4 was found for *Sargassum fluitans* (Noshkin, 1972). Considering that *S. fluitans* is transported in quantities out of the Sargasso Sea, a significant quantity of plutonium can be transferred from one water mass to another.

Large accumulations of plutonium in bulk algae samples are, in fact, reported in separate areas (Wong *et al.*, 1972). The concentrating ability of marine plants has been used to detect and estimate ^{239}Pu in the marine environment (Pillai and Mathews, 1975). Concentration variations within species, however, suggests a correlation between ^{239}Pu activity and surface area; that is, the outer-most layers tend to accumulate ^{239}Pu to a greater extent than do the inner structures (Wong *et al.*, 1972). *Pelagophyus porra*, for example, built up ^{239}Pu concentrations 3000 times that of the water, while thicker inner parts were much less active. Therefore, when different environments are compared it is often important that identical sample tissues of a given species be compared (Wong *et al.*, 1972).

The major mechanism of plutonium removal from marine systems is biological sedimentation (Bowen *et al.*, 1971; Volchok, 1974). Schell and Watters (1974) found certain conditions under which particulate plutonium could be removed from the water column and deposited in the sediments. If the majority of the plutonium is in the IV or VI valence state or is complexed with organic molecules the "mean residence time" could be many years. Mean residence time is defined as the average time an element spends in the ocean between its injection into the ocean and its incorporation in the sediments (Cherry and Shannon, 1974). If the mean residence time is long, the potential for build up in pelagic organisms would be much greater. However, in the event that plutonium has a short residence time, two possibilities occur. Continued sedimentation could effectively remove plutonium from the biosphere (Harrison and Ophel, 1975) or resuspension and redistribution at the sediment-water interface could present a continued source to the environment.

Plutonium deposited on land may be transported by erosion to streams for eventual deposition in lakes or oceans. This biogeochemical cycling is a factor to be considered in assessing the hazard potential of plutonium to man via the food web.

Analyses of beach sand and shallow water sediment (Wong *et al.*, 1970) indicate that most plutonium released to the environment near shore will concentrate in sand and silt of the sea floor and beaches. Andelman and Rozzell have proposed a biogeochemical mechanism for this process. The ability of plutonium to settle out or be carried by a flowing stream is dependent upon the interactions of various sized colloidal particles with other bottom or suspended colloidal minerals or biota. These interactions are influenced by aging, pH changes salinity fluctuations as encountered in tidal estuaries, and variations in bicarbonate and other natural complexing agents which can keep plutonium in solution (Andelman and Rozzell, 1970). To understand these processes it is useful to determine the distribution of sizes among colloidal plutonium species because these particles are likely to be encountered in water contaminated with plutonium.

Murray and Fukai (1975) used plutonium-237 to examine its adsorption-desorption characteristics with sediment particles. Plutonium (IV) particle sizes formed were comparatively homogenous and not dependent on salinity. In a river water system at pH 4, approximately 20 percent of ^{237}Pu (IV) and 30 percent of ^{237}Pu (VI) was in particulate form. This increased at pH 8 to 90 percent for ^{237}Pu (IV) and 50 percent for

^{237}Pu (VI). They estimated that under these natural conditions the major fraction of plutonium (IV) became associated with particulates, which were larger than $0.45\text{ }\mu\text{m}$, while the greater part of plutonium (VI) stayed in solution (Murray and Fukai, 1975).

Plutonium kinetics in aquatic organisms were studied by Fowler *et al.* (1975) using ^{237}Pu as a double tracer of ^{238}Pu and ^{239}Pu bioaccumulations. Shrimp and mussels accumulated plutonium slowly and the major portion was associated with the exoskeleton. These findings, similar to Ward's (1966) lobster work, indicate the potential of molting in plutonium biogeochemical cycling in the sea.

Fresh water data is much less clear. Walgren and Nelson (1974) report seasonal variations in plutonium concentrations in Lake Michigan. Their data indicate that ^{239}Pu is depleted from the surface waters during the summer months and returns during the winter to concentrations approaching those of the previous spring levels. High phytoplankton turnover during the summer months and subsequent settling of phytodetritus and fecal pellets is thought to account for this observed ^{239}Pu depletion and not accumulation by higher organisms.

By whatever mechanism, geochemical, biological, or a more complex biogeochemical mechanism, plutonium added to the ocean by any source is rapidly depleted from surface and near shore waters (Noshkin, 1972). Sediments, which represent the major depository of all plutonium released in aquatic ecosystems, need further study before there can be a complete understanding of their biological and geological significance (Pillai and Mathews, 1975).

For the food chain consisting of zooplankton to planktivore to piscivore and benthic invertebrates to bottom feeding fish there is approximately a ten-fold decrease in every successive link (Waller *et al.*, 1973). As in marine situations, the large higher trophic level freshwater predators also show lower plutonium concentrations in their gut and mussle than their prey (Livingston and Bowen, 1975). Furthermore, fish that feed high in the water column tend to have lower plutonium concentrations in their guts than benthic organisms. This decrease is consistent with the behavior of many elements that have been subject to discrimination at each step in the trophic series (Waller *et al.*, 1973).

There is, however, at least one reported case of plutonium food chain bio-magnification in marine organisms (Noshkin, 1972). The plutonium content of *Mytilus* is

directly related to the plutonium concentration in the water and is, therefore, an excellent biological indicator of plutonium pollution in the environment (Frazier and Guary, 1975; Noshkin, 1972). The $^{238}\text{Pu}/^{239}\text{Pu}$ activity ratio in *Mytilus* is more typical of values found in bottom sediments. This mussel, it is suggested, probably feeds on recently formed suspensions or bottom detritus. Starfish feeding on mussel beds were collected and analyzed. The $^{238}\text{Pu}/^{239}\text{Pu}$ activity ratio was the same as the average mussel value. This indicates that plutonium enters the starfish by way of the prey rather than the environment. These observations led Noshkin (1972) to suggest that plutonium concentrations may increase along a simple food chain.

Noshkin (1972) concludes that while fish flesh does not represent an important transfer vector of environmental plutonium into human food chains, there are aspects of fish ecology and physiology that result in plutonium reconcentrations in the rarely eaten parts of the organism. Therefore, such food items as anchovies, sardines or canned salmon which are consumed whole, and fish protein concentrate which is likely to increase in usage in the future, will represent important vectors of plutonium into humans through the food chain.

Gromov and Spitsyn (1974a) investigated the assimilability of plutonium by phytoplankton. Green algae cultures, *Pladymonas viridus*, were spiked with $0.02 \text{ mg} \cdot \text{l}^{-1}$ ($\sim 5 \times 10^{13} \text{ Pu atoms} \cdot \text{ml}^{-1}$). Algae "rapidly assimilated" plutonium and reached equilibrium within 20 hours. Bacterial decomposition of the algae reportedly released the adsorbed plutonium and changed its physiochemical state to one in equilibrium with the environment. This biological cycling was suggested as an important mechanism for plutonium migration in the ocean. In a companion paper (Gromov and Spitsyn, 1974b), this change from an ionic form to an organic complex changed plutonium sorption by bottom sediments and suspensions.

Zlobin (1967) studied the accumulation of uranium and plutonium by northern marine algae, *Chorda filum*, *Monostroma fuscum* and *Laminaria saccharina*. These species, in decreasing order, were found to concentrate plutonium to a considerably greater degree than uranium. This was thought to be due to the presence of plutonium predominantly as solid particles. The data indicated that phytoplankton possess a more pronounced ability to accumulate artificially introduced plutonium than naturally present uranium. Factors suggested for the differences in interspecies accumulation were: 1) adsorption properties of algae; 2) magnitude of the charge on the cell; and 3) rates of exchange between ionic uranium,

concentrated from water, and the uranium (or plutonium) subsequently introduced into the water.

Havlik and Robertson (1971), working with radium-226, found the species of algae was the dominant factor in radium accumulation. Further, algae were broadly divided into two groups: 1) those which absorb radium into the cells; and 2) those which accumulate radium on the cellular surface (Havlik, 1971).

Sodium cyanide was found to significantly inhibit ^{239}Pu accumulation by the marine alga *Ascophyllum nodosum* (Zlobin and Mokuau, 1970). Blocking the cytochrome system with cyanide demonstrated involvement of the active transport mechanism in plutonium accumulation (Zlobin, 1971). That an important mechanism as cellular respiration participated in plutonium uptake indicated, at least in part, that plutonium accumulation was an active process and not entirely a surface phenomenon.

Giesy and Paine (1977) demonstrated cell density to be a factor in americium-241 accumulation by green algae and bacteria. They noted an inverse relationship between concentration factors and cell densities of *S. obliquus*, *S. capricornatum* and *C. pyrenoidosa*. Further, on a uptake per gram basis the greatest accumulation was by the largest cells, *S. obliquus*, followed by algae of intermediate size, *S. capricornatum*. The smallest species

investigated, *C. pyrenoidosa*, showed the lowest ^{241}Am uptake. Other mechanisms, such as those involving surface binding sites, were suggested to be more relevant to ^{241}Am uptake than simple surface to volume ratio accumulation (Giesy and Paine, 1977).

MATERIALS AND METHODS

Axenic cultures of *Scenedesmus obliquus* (Türp) Kütz obtained from the Indiana culture collection (Strain 1592) were used in these experiments. Algal Assay Procedure (AAP) nutrient medium (Anon, 1971) was sterilized to 20 psi for 45 minutes in 250 ml wide-mouth Erlenmeyer flasks. Flasks containing 150 ml of the solution (Table 1) were flamed in a laminar flow hood to prevent extraneous contamination. A one-ml aliquot of the axenic culture was introduced into each flask. Flasks were stoppered with two-hole No. 2 stoppers. In one hole a Pasteur-type pipette was inserted for culture aeration. Pipette tips were flamed and bent slightly allowing tips to reach the bottom of the flask. Flasks were maintained at 45° to facilitate culture aeration. Teflon^R tubes connected pipettes to aquarium-type gang valves. In-line 45-μ membrane filters were connected to these tubes, preventing particulate matter from contaminating the cultures. Each of four gang valves could support five cultures and were connected to an aquarium pump by polyethylene tubing. Inserted into the second stopper hole was a 5 cm glass tube containing a membrane filter to prevent microbial contaminants from entering the flask through the exhaust hole. One-cm

Table 1. Modified synthetic algal assay procedure (AAP) medium developed by the National Eutrophication Research Program, Environmental Protection Agency (Anon., 1971).

<i>Macronutrients</i>			
<i>Compound</i>	<i>Concentration mg/l</i>	<i>Element</i>	<i>Concentration mg/l</i>
NaNO ₂	51.000	N	8.400
K ₂ HPO ₄	3.132	P	0.372
MgCl ₂	11.400	Mg	5.808
MgSO ₄ • 7H ₂ O	29.400	S	3.822
CaCl ₂ • 2H ₂ O	8.820	C	4.286
NaHCO ₃	30.000	Ca	2.404
		Na	22.002
		K	0.938
<i>Micronutrients</i>			
<i>Compound</i>	<i>Concentration µg/l</i>	<i>Element</i>	<i>Concentration µg/l</i>
H ₃ BO ₃	371.040	B	64.920
MnCl ₂	528.528	Mn	230.748
ZnCl ₂	65.418	Zn	31.382
CoCl ₂	1.560	Cu	0.708
CuCl ₂	0.018	Cu	0.008
Na ₂ MoO ₄ • 2H ₂ O	14.520	Mo	5.756
FeCl ₃	192.000	Fe	99.153
Na ₂ EDTA • 2H ₂ O	600.000	Na	73.800

holes were drilled through each stopper and plugged with a Vacutaner^R plug, through which sterile aliquots could be removed to monitor growth. Aeration apparatus and Erlenmeyer flasks containing AAP were autoclaved before culture inoculation.

Cultures (shown in Figure 1) were maintained at 25°C in a controlled environment chamber and illuminated on a 12-hour cycle by balanced spectrum Grow Lux^R fluorescent lamps.

Stock cultures (150-ml, 5×10^5 cells·ml⁻¹) were transferred to separate 4000-ml Erlenmeyer flasks containing 3500-ml sterile AAP. These five large experimental cultures were maintained under the same conditions and with similar apparatus as the smaller stock cultures. Culture transfers were carried out to obtain more uniform algal growth rates than would have been possible had such large cultures been initiated from only a few cells.

Culture densities were determined by microscopic examination using calibrated phytoplankton counting chambers. Ten grid counts were averaged and converted to cells·ml⁻¹. In early stationary phase, cells were centrifuged from stocks using a Sorvall SS-1 continuous flow centrifuge equipped with a KSB continuous flow system. The system was presterilized with ethanol and rinsed with sterile water. Seventeen liters of algal

Figure 1. Photograph of algal culture apparatus in environmental chamber; note aquarium gang valves, air delivery system and in-line 0.45 μ membrane filters.

Figure 1.

suspension were concentrated in 500 ml fresh AAP medium. This suspension was again centrifuged at 8×10^3 rpm for 10 minutes over 28 percent by weight bis-2(ethylhexyl) sebacate in N-butylphthalate to separate viable cells from dead cells and cell fragments. The recovered cells were resuspended in 350 ml fresh sterile AAP. The resulting cell density was 1×10^7 cells $\cdot$ ml $^{-1}$. This stock culture was used in uptake studies and maintained in the dark.

Four experimental groups in a 2×2 design with five replications were studied at three plutonium concentrations. Treatments consisting of uniform algal cultures to which were added plutonium nitrate solutions of: ^{238}Pu in the IV valence state ($^{238}\text{Pu}^{+4}$); 2) ^{238}Pu in the VI valence state ($^{238}\text{Pu}^{+6}$); 3) ^{239}Pu in the IV valence state ($^{239}\text{Pu}^{+4}$); and 4) ^{239}Pu in the VI valence state ($^{239}\text{Pu}^{+6}$). In addition there was one control blank per treatment and concentration containing all components of the experimental groups except algae. Controls were handled similar to the experimental groups in order to monitor contamination during the separation procedure.

Three ^{238}Pu and ^{239}Pu concentrations in both the +4 and +6 valence state were obtained from the Analytical Chemistry Division of the Savannah River Laboratory. Nominal ^{238}Pu concentrations were: 1.3×10^{-13} ; 4×10^{-12} ; 1.3×10^{-11} mg $\cdot$ ml $^{-1}$ (2.50; 75.0; 250.0 pCi $\cdot$ ml $^{-1}$).

Plutonium-239 concentrations were: 4×10^{-12} ; 1.3×10^{-11} ; $1.3 \times 10^{-10} \text{ mg} \cdot \text{ml}^{-1}$ (.25; .90; 9.00 pCi $\cdot\text{ml}^{-1}$). Actual plutonium concentrations in experimental flasks are listed in Table 2. A ^{239}Pu concentration comparable to the lowest ^{238}Pu concentration activity is approximately 0.4 d/m which is below detectability of the counting apparatus. Plutonium-238 and ^{239}Pu therefore, did not overlap at this level. There was no attempt to achieve a ^{238}Pu concentration similar to the highest ^{239}Pu level, because that high activity would present counting problems and safety hazards. Thus the experiment consisted of 60 experimental units and 12 control blanks. However, because the centrifuge only had space for eight samples these were orthogonally randomly blocked into groups of eight and spaced 1-1/2 hours apart.

Assimilation studies of plutonium isotopes by *S. obliquus* were carried out in 250-ml Erlenmeyer flasks containing 93 ml sterile AAP. One ml of 1N HNO_3 plutonium solution was pipetted into each flask, the pH adjusted to 4.5-5.5 with 0.9 ml 1N NaOH and five ml of the algal concentrate introduced resulting in a total volume of 100 ml and algal cell density of $\sim 5.5 \times 10^5 \text{ cells} \cdot \text{ml}^{-1}$. The experiment was performed over three days using a different plutonium concentration each day, and the algal concentration declined slightly over this period. Experimental flasks containing the plutonium, AAP and algae were

Table 2. Plutonium Nitrate Concentrations in Experimental Flasks

Isotope and Oxidation State	Concentration (atoms·ml ⁻¹)			
²³⁸ Pu ⁺⁴	3.25 × 10 ⁸ (n=5)	9.15 × 10 ⁹ (n=5)	2.71 × 10 ¹⁰ (n=5)	
²³⁸ Pu ⁺⁶	6.87 × 10 (n=5)	1.11 × 10 ¹⁰ (n=5)	3.78 × 10 ¹⁰ (n=5)	
²³⁹ Pu ⁺⁴		1.11 × 10 ¹⁰ (n=4)	3.87 × 10 ¹⁰ (n=5)	3.78 × 10 ¹¹ (n=3)
²³⁹ Pu ⁺⁶		1.04 × 10 ¹⁰ (n=3)	4.61 × 10 ¹⁰ (n=5)	3.81 × 10 ¹¹ (n=5)

n = number of replicate flasks

incubated four hours. Two 5-ml aliquots were taken for cell density determinations and plutonium plating loss during the four-hour experimental period. One 10-ml aliquot was taken from each control blank as a check for plating loss determination.

The remaining culture in each flask was transferred in 30-ml aliquots to a polyethylene centrifuge tube and centrifuged at 1.3×10^4 rpm for 15 minutes over 28 percent by weight bis-2(ethylhexyl)sebacate in N-butylphthalate to separate algal cells from solution (Giesy and Paine, 1977). Three centrifugations were required to separate the 90-ml of culture remaining in the flask. Following each centrifugation, supernatant from each centrifuge tube was aspirated into a companion 100-ml polyethylene bottle and maintained. The algal cells were accumulated in the centrifuge tube until all the culture from the flask was centrifuged. Algal cells and bis-2(ethylhexyl)sebacate were washed into test tubes with acetone and stoppered.

Analytical Procedure

Early work demonstrated liquid scintillation as an effective method of alpha determination in biological samples (Toribara *et al.*, 1962). Liquid scintillation counting has a number of inherent advantages (Horrocks, 1964) including ease of sample preparation and 100 percent

counting efficiency. When coupled to a multichannel analyzer, allowing peak discrimination, difficulties normally presented by background variations (Thorngate *et al.*, 1974) and quenching (McKlveen and McDowell, 1975) can be greatly reduced.

Two liquid scintillation systems were used in this environment: 1) Nuclear Chicago liquid scintillation counter interfaced to an Ortec multichannel analyzer; and 2) Packard Tri-Carb coupled to a Technical Measurements Company multichannel analyzer. Although these systems did not have the excellent pulse resolution characteristics of custom experimental systems (McKlveen and McDowell, 1975), they detected low activity ^{238}Pu and ^{239}Pu samples quite effectively.

Radiochemical analysis involved a combination of wet and dry ashing (Boni, A. L., personal communication, 1976). One milliliter aliquots of ^{237}Pu were added to each sample and samples were ashed to dryness in 10-ml of 8N HNO_3 . Following dry ashing at 500°C in a muffle furnace for six hours, samples were twice dissolved in 15-ml of 8N HNO_3 and taken to dryness. The final residue was dissolved in 3-ml of hot 1N HNO_3 and a 2-ml aliquot pipetted into a liquid scintillation vial containing 20-ml of scintillation solution, Aquasol-2 (New England Nuclear). Samples were counted in a liquid

scintillation counter for 10-1000 minutes depending upon sample activity.

Plutonium-237 tracer concentration in each sample was determined using a 20 cm NaI well-type detector. Taking the small ^{237}Pu alpha contribution (see Table 3) into account the total sample plutonium was calculated:

$$\text{Total sample plutonium (d/m)} = \left[s_a^{\text{Pu}_\alpha} - \frac{{}^{237}\text{Pu}_\alpha}{s_t^{{}^{237}\text{Pu}_\gamma}} s_a^{\text{Pu}_\gamma} \right] \frac{{}^{237}\text{Pu}_\gamma}{s_a^{{}^{237}\text{Pu}_\gamma}}$$

where $s_a^{\text{Pu}_\alpha}$ and $s_t^{{}^{237}\text{Pu}_\alpha}$ are the net alpha counts of the sample and the ^{237}Pu standard in counts $\cdot\text{min}^{-1}$; $s_a^{\text{Pu}_\gamma}$ and $s_t^{{}^{237}\text{Pu}_\gamma}$ are the net gamma counts of the sample and ^{237}Pu standard in counts $\cdot\text{min}^{-1}$.

The ^{237}Pu product used in this experiment was produced at the Oak Ridge National Laboratory (ORNL). Plutonium-237 decays by electron capture (99%), with little (0.002%) alpha particle emission (Hoffman and Dropesky, 1958). The x-rays of ^{237}Np resulting from plutonium decay are reasonably energetic (100 keV complex) to be measured on NaI scintillation systems.

Plutonium-237 has been used previously to model ^{239}Pu behavior in the environment (Murray and Fuikai, 1975; Trabakla and Eyman, 1976) and uptake and elimination kinetics in animals (Todd and Logan, 1958; Bair *et al.*, 1974). However, much higher specific activity (Ci/g) of

Table 3. Analysis of Plutonium-237 Product as of November 4, 1976

^{237}Pu	1.4 $\mu\text{Ci/ml}$
Solution	0.5 <u>M</u> HCl
^{67}Ga (78 hr)	0.8 nCi/ μCi ^{237}Pu
Gross Alpha	1.8 nCi/ μCi ^{237}Pu
Alpha Components	4.9 MeV ~9.2%
	5.3 MeV ~13.7%
	5.7 MeV ~71.1%

^{237}Pu compared with ^{239}Pu clouded interpretations of much of the data (Moghissi and Carter, 1975). This difference in specific activity was exploited in this study by using ^{237}Pu as a tracer for chemical yield.

Kressin *et al.*, (1975) compared the use of ^{236}Pu and ^{242}Pu , both alpha emitters, as tracers. Plutonium-242 changed the background for ^{238}Pu and ^{239}Pu significantly less than ^{236}Pu and was, therefore, considered the superior tracer. This applied to high resolution alpha spectroscopy systems, and using these tracers with either low resolution systems or gross alpha systems involved other considerations. In this case, samples must be divided into equal aliquots with one receiving the tracer. This further reduced what may have been an already low activity sample. Difference in sample activity was due to the tracer, and the question of increased background became academic. The use of ^{237}Pu as a tracer eliminated the need to split samples, but it required each sample be counted twice and the alpha component be considered.

Data were converted from alpha decays $\cdot\text{min}^{-1}$ of the sample to plutonium atoms by:

$$\text{Pu atoms} = \frac{d/m}{(\text{SA})(\text{AT})(2.22 \times 10^{12}) \text{ d/m}\cdot\text{Ci}^{-1}} \cdot (\text{AN})$$

where:

d/m = alpha activity sample (decays/min),

SA = specific activity of isotope (curies $\cdot$ gram $^{-1}$),

AT = atomic weight of isotope (g)

$AN = (6.023 \times 10^{23} \text{ atoms} \cdot \text{gram atom}^{-1})$

This facilitated direct comparison of ^{238}Pu and ^{239}Pu masses. Plutonium in solution is reported as plutonium atoms $\cdot$ ml $^{-1}$ and plutonium associated with algal cells is reported as plutonium atoms $\cdot$ cell $^{-1}$. Thus the "pCi/gram" term was eliminated as it presented a distorted representation of isotopic behavior when comparing isotopes of widely differing half-lives, such as ^{238}Pu (88 years) and ^{239}Pu (24,000 years).

The liquid scintillation systems used had backgrounds of 23 cpm with 2SE minimum detectability of 0.5 cpm. The use of liquid scintillation procedures for plutonium analysis had the following advantages over an alternative analysis method that used electrodeposition of plutonium on a platinum disk following separation of plutonium from other ions (Harley, 1970): 1) it was rapid, allowing preparation of up to 50 samples a week; 2) its simplicity eliminates the need for sophisticated equipment; 3) it is relatively inexpensive; 4) liquid scintillation allows approximately 100 percent counting efficiency versus 25-28 percent for electrodeposition (LeRoy, J. H., personal communication, 1977).

RESULTS AND DISCUSSION

The experimental results of plutonium uptake by the green algae *S. obliquus*, as a function of isotope and chemical valence state, are summarized in Figure 2. Comparisons of algal uptake were made using two concentrations at two oxidation states and two plutonium isotopes.

The amount of $^{238}\text{Pu}^{+4}$ and $^{238}\text{Pu}^{+6}$ associated with algal cells was directly proportional to ^{238}Pu concentration in the medium (Figure 2). There was no significant difference (two standard errors of the mean; $p = 0.05$) between the algal sorption of ^{238}Pu or ^{239}Pu due to oxidation state at the three concentrations studied. There was no significant difference between the algal uptake of ^{238}Pu and ^{239}Pu at similar concentrations.

Before proceeding to conclusions about algal uptake of plutonium as a function of valence state and isotope, a number of factors need be considered, including the plating out of plutonium and the complexities of plutonium chemistry. Plutonium can co-exist in solution at all four oxidation states between Pu^{+3} and Pu^{+6} (Bagnall, 1972), and the question of Pu^{+4} and Pu^{+6} equilibrating to the same form, or forms, must be addressed. What was

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Figure 2. *S. obliquus* uptake curves of four plutonium forms. Each point represents the mean of five replications reported as $\log_{10}$ plutonium atoms $\cdot$ cell $^{-1}$ with the error bars representing one standard error of the mean.

Figure 2.

known in this study was the original plutonium form preceeding addition to the medium. It is not likely, however, that both Pu^{+4} and Pu^{+6} solutions converted in exactly the same manner, because at equilibrium the Pu^{+4} disproportionation reaction to Pu^{+3} and Pu^{+6} is not the same as the Pu^{+6} reduction reaction to Pu^{+4} (Kasha, 1949). Therefore, changes in plutonium form did not account for the interpretation of no difference in algal sorption of either plutonium form.

Plutonium is also known to "plate out" on vessel walls and be effectively removed from solution. However, when each entire solution and its associated algal fraction were assayed and taken together, there was a 96.5 percent mass balance. Another effort was made to determine the extent of this phenomenon, i.e., five-ml aliquots were removed from each sample, prior to algal centrifugation, and analyzed for plutonium content. However, the resultant data were too variable for an accurate assessment of this question.

The plutonium concentrations studied here are relevant to levels encountered in the environment. The plutonium concentration reported in the Savannah River is approximately 10^7 atoms $\cdot\text{ml}^{-1}$ (Anon., 1976). This level is just below the lowest ^{238}Pu level studied (10^8 atoms $\cdot\text{ml}^{-1}$). The maximum permissible ^{238}Pu concentration

in drinking water is 10^{11} atoms $\cdot$ ml $^{-1}$ (Hayes *et al.*, 1975), and falls between the higher overlapping ^{238}Pu and ^{239}Pu levels and the highest ^{239}Pu level studied.

Since the algal concentration in the experiment was 5×10^5 cells $\cdot$ ml $^{-1}$, it can be approximated, from Figure 2, that nearly 20 percent of the plutonium in solution was associated with the algal fraction. This leads to a number of implications, primarily that plutonium associated with algae in an environment can be estimated by determining the algal density and the plutonium concentration of the system. In addition, a considerable portion of plutonium in water to be treated can be removed simply by removing the algae. Further, plutonium behavior in the environment can be modeled using either ^{238}Pu or ^{239}Pu in either the Pu^{+4} or Pu^{+6} oxidation state.

SUMMARY AND CONCLUSIONS

This study was designed to determine the effect of plutonium chemical valence state on the availability of small concentrations of ^{238}Pu and ^{239}Pu to algae. While there have been extensive surveys of plutonium source terms and distribution in aquatic ecosystems, experimental work on availability of plutonium to algae is quite limited, particularly at environmental levels.

The uptake experiments involved the green alga *Scenedesmus obliquus*, grown in batch cultures. Plutonium concentrations accumulated by this alga were linearly related to plutonium concentrations. There was no significant difference ($p = 0.05$) in algal plutonium accumulations, on a mass basis, of either ^{238}Pu or ^{239}Pu in either Pu^{+4} or Pu^{+6} oxidation state at the concentrations studied.

The sample preparation method used was simple and inexpensive and had a number of advantages over the electrodeposition method. The modified liquid scintillation counting systems were suitable for determining the plutonium content of the samples and were accurate over a wide range of sample activities. In addition, ^{237}Pu was an effective tracer for plutonium chemical recovery.

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