

Experimental studies on the accumulation of polonium-210 by marine phytoplankton

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Abstract

Bioaccumulation of polonium-210 ($t_{1/2} = 138$ d) in marine phytoplankton can introduce this naturally occurring radioisotope into food chains, where it accounts for most of the radiation dose to marine organisms and to human consumers of seafood. Moreover, this isotope could be useful as a tracer of the flux of organic matter in ocean surface waters. We performed laboratory experiments with eight algal species representing six algal divisions to quantify the uptake, cellular partitioning, and retention of ^{210}Po by algae. Biological uptake was unaffected by temperature or light, and volume concentration factors (VCFs) for these algal species ranged between 0.5 and 3.0×10^4 . Interspecific differences in VCFs could be explained by considering the surface area to volume ratios of the cells and cellular protein content. Once associated with the cells, between 30 and 60% of the total cellular ^{210}Po was in the cytoplasm of the different species. ^{210}Po was not irreversibly bound to the cells but displayed a biological half-life of ~ 23 d. Because ^{210}Po associates appreciably with organic matter inside cells, unlike other particle-reactive nonessential metals, it could have promise as a tracer of sinking organic matter in the ocean.

Polonium-210 is both a major source of natural radiation to marine organisms as well as a potential chemical tracer for organic matter sinking out of ocean surface waters. In order to better evaluate the usefulness of ^{210}Po as an organic matter tracer and to comprehend its bioconcentration in marine food webs, the association of this element with marine phytoplankton needs to be better understood. There have been few controlled experiments in which the uptake of ^{210}Po by plankton, in particular phytoplankton, has been examined (Fisher et al. 1983b). Studies on the partitioning of ^{210}Po between particulate and dissolved phases have shown that this element, like Pb, is very particle-reactive, with reported K_d values for various types of particles in the range of 10^3 – 10^5 (Carvalho 1997; Nozaki et al. 1997; Hong et al. 1999). Results of earlier studies suggest that ^{210}Po also becomes incorporated into the cytoplasm of some species of phytoplankton (Fisher et al. 1983b) and bacteria (Cherrier et al. 1995; LaRock et al. 1996), where, unlike ^{210}Pb , its partitioning is similar to that of protein and sulfur within the cell. Thus, there is evidence that potentially two pools of ^{210}Po are associated with cells: a surface-bound pool and an internal pool. The polonium in the internal pool's association with cytoplasm and protein could result in efficient assimilation of ^{210}Po by grazing zooplankton (Reinfelder and Fisher 1991) that, in turn, could lead to its bioconcentration in marine food webs. The external pool, in contrast, could be expected to behave more like other surface-bound particle-reactive radionuclides such as ^{210}Pb and ^{234}Th , which are not assimilated in animal tissue and sink in fecal material (Fisher and Reinfelder 1995).

The final alpha-emitting product of the radioactive decay of ^{238}U , ^{210}Po ($t_{1/2} = 138$ d), is produced by the beta decay

of ^{210}Pb ($t_{1/2} = 22$ yr) via the short-lived intermediate ^{210}Bi ($t_{1/2} = 5$ d). ^{210}Pb is delivered to the ocean largely by atmospheric deposition (Shannon et al. 1970; Carvalho 1997); thus, its input into the ocean is not constant. Most ^{210}Po in the ocean is produced by the in situ decay of ^{210}Pb , although point sources near phosphate ore-processing operations and seamount volcanoes could contribute to higher polonium concentrations regionally (LaRock et al. 1996; Rubin 1997). Polonium is found in subtrace concentrations in surface seawater, on the order of 10^{-20} mol L^{-1} or 0.3–3.0 mBq L^{-1} (Cochran et al. 1983; Hong et al. 1999). The vertical profile of dissolved polonium is like that of nutrient elements: the concentration is lowest at the surface, despite the atmospheric source from wet and dry deposition, and rises slightly with depth (Cochran et al. 1983). ^{210}Po is quickly scavenged out of the surface layer, leading to estimated residence times in surface waters of approximately 0.6 yr (Bacon et al. 1976; Cochran et al. 1983), a value that is about half its residence time in deeper waters (Kadko et al. 1987).

^{210}Po concentrates appreciably in marine animals (Heyraud and Cherry 1979; Bulman et al. 1995; Dahlgard 1996; Durand et al. 1999), providing the largest radiation doses to aquatic organisms. The concentration of ^{210}Po is ≥ 148 mBq g^{-1} in phytoplankton (Cherry 1964), 3,145 mBq g^{-1} in shrimp hepatopancreas (Cherry and Heyraud 1981), ≤ 700 and $\leq 1,026$ mBq g^{-1} in mussel soft tissues (Germain et al. 1995) and hepatopancreas (Stepnowski and Skwarzec 2000), respectively, and ≤ 262 mBq g^{-1} in fish (pyloric caecum, Clulow et al. 1998). In contrast, humans have an average ^{210}Po concentration of 0.148 mBq g^{-1} , yielding a dose that is up to two orders of magnitude lower than those received by marine animals (Cherry 1964). In fact, studies in the Irish Sea within the path of the discharges from the Sellafield nuclear fuel reprocessing plant have found higher doses to organisms from ^{210}Po than from anthropogenic radionuclides (Pentreath and Allington 1988). Besides the exposure to the animals themselves, the highly enriched concentrations of ^{210}Po in marine organisms can contribute significantly to human radioactivity exposure through seafood consumption (Bulman et al. 1995; Dahlgard 1996; Carvalho 1997). Un-

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Understanding the biological interactions of polonium is thus important in risk assessments that consider the health of ecosystems and public health.

^{210}Po has been used to trace particle transfer (Radakovitch et al. 1999), ocean circulation (Moore and Smith 1986; Carvalho 1997), and vertical flux of particulate matter in the ocean (Nozaki et al. 1997, 1998; Friedrich and Rutgers van der Loeff 2002). Like uranium and thorium, radioactive lead and polonium should come to secular equilibrium, in this case on a time scale of roughly 2 yr (assuming steady state), but the two are often in disequilibrium in the surface layer of the ocean (Cochran et al. 1983; Nozaki et al. 1997, 1998). In fact in the upper ocean, the average dissolved $^{210}\text{Po}/^{210}\text{Pb}$ activity ratio is near 0.5 (Hong et al. 1999), implying that ^{210}Po is preferentially removed from surface waters because of the different affinities of these elements for particulate matter. Nozaki et al. (1998) found a strong correlation between the dissolved ^{210}Po removal rate constant and chlorophyll *a* concentration, but no similar correlation for ^{210}Pb , suggesting a link between phytoplankton abundance and ^{210}Po removal.

Here, we present results of a study to assess more fully the uptake and distribution of ^{210}Po in marine phytoplankton. We compared the concentration of ^{210}Po by phytoplankton species that differed in size, surface characteristics, and protein content to investigate the relationship between these parameters and ^{210}Po uptake. We also examined the reversibility of the ^{210}Po -phytoplankton associations to further examine the consequences of the partitioning of polonium in the cells. Understanding the relationship between ^{210}Po and various cellular components can provide a mechanistic basis for evaluating differences in the flux of this radionuclide, its possible concentration in higher trophic levels, and its potential use as a tracer of organic carbon flux in the sea.

Materials and methods

^{210}Po uptake by eight marine algal species was determined in a series of laboratory experiments. The species studied were the centric diatom *Thalassiosira pseudonana* (clone 3H), the chlorophytes *Dunaliella tertiolecta* (DUN) and *Chlorella autotrophica* (CCMP 243), the coccolithophore *Emiliania huxleyi* (CCMP 2112), the prymnesiophyte *Isochrysis galbana* (ISO), the cryptophyte *Rhodomonas salina* (CCMP 1319), the prasinophyte *Tetraselmis levis* (PLATY 1), and the dinoflagellate *Heterocapsa triquetra* (OB 21019305). All inocula came from axenic stocks; cultures were handled aseptically throughout the experiments. Cultures were maintained in sterile-filtered (0.2 μm Nuclepore polycarbonate membrane) surface seawater collected 8 km off Southampton, New York, and enriched with f/2 nutrients (Guillard and Ryther 1962). Experimental inocula were harvested either by resuspension off Nuclepore membranes or by centrifugation at $3,000 \times g$ (*R. salina* and *I. galbana*) from stock cultures in late log phase and grown in sterile 200-ml ground glass-stoppered erlenmeyer flasks, each containing 100 ml of filtered growth medium. Control treatments were identical to algal cultures, except the growth medium remained uninoculated. We also measured the bind-

ing of ^{210}Po to sterilized acid-washed glass beads (5–15 μm , median diameter 10 μm) to assess the adsorption of ^{210}Po onto inorganic mineral surfaces. Suspensions of the glass beads were treated identically to the algal cultures. All treatments were run in triplicate flasks and cell counts were made daily.

Cultures were incubated at 15°C under cool white fluorescent lamps producing about 100 μmol quanta $\text{m}^{-2} \text{s}^{-1}$ at the culture surface (14:10 light:dark [LD]). The initial biomass (dry wt) in the cultures ranged from 100 to 500 μg C L^{-1} , and growth was monitored periodically throughout the experiments using a Coulter Counter (Multisizer II) and checked microscopically with a hemacytometer. Cell volumes, also measured using the Coulter Counter, ranged between 26 (*C. autotrophica*) and 2,693 μm^3 (*H. triquetra*) (Table 1). Surface areas, calculated using microscopy and geometric formulas, ranged from 42 to 979 μm^2 . Surface area to volume ratios ranged between 0.75 and 2.0 μm^{-1} (Table 1). Dimensions of the glass beads fell in the middle of all ranges and were very similar to the values for *T. pseudonana*.

In addition to analyzing cells for ^{210}Po content, the cells were analyzed for protein content using the Sigma Aldrich BCA protocol (Smith et al. 1985) for low-protein content measured against a standard of pure bovine serum albumin. When normalized to cell volume, the protein content of cells ranged between 0.05 (*H. triquetra*) and 0.15 pg μm^{-3} (*R. salina*) (Table 1). Our measurements were comparable to those determined in earlier work (Fisher et al. 1983a, 1991); for example, we found 7.4 pg protein cell^{-1} for *T. pseudonana*, and Fisher et al. (1983b) found 7.6 pg protein in each cell. The protein content of the glass beads, which might have sorbed to the bead surfaces from ambient seawater, was below detection. The C:N ratio of the cells, determined with a known quantity of cells on precombusted GFF filters using a Carlo Erba model 1602 CNS analyzer standardized with sulphanilamide, ranged from 6.9 to 7.5 (Table 1).

^{210}Po in 2 N HCl (Isotope Products) was added in micro-liter amounts to yield an initial ^{210}Po concentration of 60–90 Bq ml^{-1} (7.7–11.6 nmol L^{-1}) in growth medium. ^{210}Po was added at least 24 h prior to the addition of cells or glass beads to allow equilibration between different dissolved fractions; Ultrapur NaOH was added to buffer the acid in the ^{210}Po stock. The pH of the growth medium was unaffected by the isotope addition and increased to as much as 8.3 following algal growth. No attempt was made to characterize the speciation of the radioisotope after its introduction to the seawater, but initial time series experiments indicated that equilibrium was reached within 24 h.

T. pseudonana was also grown in f/2 without any ethylenediaminetetraacetic acid (EDTA), Cu, or Zn to test the effects of the chelator on the bioavailability of ^{210}Po . Additionally, we exposed this diatom to three concentrations of ^{210}Po (1, 5, and 10 nmol L^{-1}) to test the effects of metal or radiotoxicity, because the concentrations of ^{210}Po in the experiment far exceeded those in seawater. To test the effects of temperature and light on ^{210}Po uptake, *T. pseudonana* was exposed to ^{210}Po at 4 and 15°C in a 14:10 LD cycle and in constant light and constant darkness at 15°C. For this ex-

Table 1. Characteristics of the glass beads and eight species of phytoplankton used in these experiments. All numbers are means from three replicate cultures. The CV for every value other than the VCFs in this table is <10%. See text for explanation of VCF calculations, which are shown as means \pm 1 SD. The detection limit of the BCA protein analysis was 0.77 pg cell⁻¹.

	Beads	<i>C. autotrophica</i>	<i>D. tertiolecta</i>	<i>E. huxleyi</i>	<i>I. galbana</i>	<i>H. triquetra</i>	<i>R. salina</i>	<i>T. levis</i>	<i>T. pseudonana</i>
SA (μm^2)	85	42	198	81	58	977	173	222	82
Vol (μm^3)	72	26	186	68	41	2,693	189	297	69
SA/Vol (μm^{-1})	1.18	1.63	1.06	1.18	1.40	0.36	0.91	0.75	1.18
C:N	nd	7.2	7.4	7.0	7.3	7.0	7.5	7.1	7.2
Protein/cell (pg cell ⁻¹)	nd	1.3	11.2	7.0	3.2	125.6	29.2	29.4	7.3
Protein/Vol (pg μm^{-3})	nd	0.09	0.06	0.10	0.08	0.05	0.15	0.10	0.11
Po/cell (amol cell ⁻¹)	2	2.5	3	3.6	3	25	2.2	9	4
Po/Vol (amol μm^{-3})	0.02	0.10	0.02	0.05	0.01	0.01	0.04	0.03	0.06
% in cytoplasm	nd	58	52	31	43	42	28	45	39
VCF ($\times 10^3$)	9.6 (± 2.2)	26.7 (± 4.9)	9.9 (± 2.1)	17.2 (± 2.5)	17.0 (± 3.3)	6.1 (± 1.5)	16.7 (± 2.7)	14.2 (± 1.7)	19.5 (± 2.6)

periment, ²¹⁰Po uptake was evaluated over a 1-d period with more frequent sampling at the beginning of the experiment.

The partitioning of ²¹⁰Po was monitored for up to 5 d following methods described in Fisher et al. (1983a). Samples included 1.0- μm Nuclepore membranes containing filtered cells (from 5 ml), washed with filtered seawater containing no added ²¹⁰Po, and 1-ml aliquots of the ²¹⁰Po-labeled cell suspensions. The radioactive samples were placed in scintillation vials with 10 ml of Ultima Gold XR scintillation cocktail. Samples were shaken vigorously to promote homogenization before being placed in a Packard Tri-Carb 2100TR liquid scintillation counter. Samples were counted for 5 min, yielding propagated counting errors of <5%; the counting efficiency was calculated to be near 99% using a known standard of ²¹⁰Po. All samples were counted with appropriate blanks and standards and were corrected for radioactive decay.

For all species, additional cells were exposed to the ²¹⁰Po f/2 solution at the same time as the uptake experiments for fractionation and localization of ²¹⁰Po in the cells. All species were exposed to ²¹⁰Po for at least three cell divisions to uniformly label the cells, filtered onto 1- μm membranes, and resuspended in distilled water, causing partial cell lysis. This suspension of cells was then placed in a sonic bath for 5 to 10 min until visual inspection confirmed that all the cells were lysed. The resulting suspension was then centrifuged in 25-ml Pyrex vessels at 10,000 \times g for 15 min at 4°C. A cohesive pellet was formed for all species. The supernatant, containing the cytoplasmic fraction of the lysed cells (Sheeler 1981; Fisher et al. 1983b), was carefully pipetted out of the centrifuge tube without disturbing the pellet. The supernatant was then added to a scintillation vial and counted. The pellet, which contained membrane-bound and cell wall-bound ²¹⁰Po, was counted separately.

Following uptake of ²¹⁰Po by the algal cells or glass beads, we assessed the reversibility of the binding of ²¹⁰Po to the particles by resuspending the radiolabeled particles in filtered seawater containing no added ²¹⁰Po, following protocols described in Fisher and Went (1993), in which the desorption of the radioisotope was measured over time. Cells were resuspended at the same cell densities as at the end of the ²¹⁰Po exposure period and were incubated at 4°C for 24 h in the dark to keep the cell density constant. Loss experiments with all species were run in duplicate and sampled periodically.

To investigate the loss of polonium to volatilization, a possible problem in such experiments (Momoshima et al. 2001), the liquid remaining in the flasks at the end of the experiments was decanted, and the empty flasks were rinsed four times with 10% HCl and once with 6 N HCl. These acid rinses were collected and analyzed to account for sorption to flask walls. The counts from these rinses were added to the counts calculated from aliquots of the total solution and compared to the original amount of ²¹⁰Po added to each flask to assess ²¹⁰Po distribution in the flasks.

Results

Algal growth during ²¹⁰Po exposure is shown in Fig. 1. As the cells grew, they removed increasingly more ²¹⁰Po

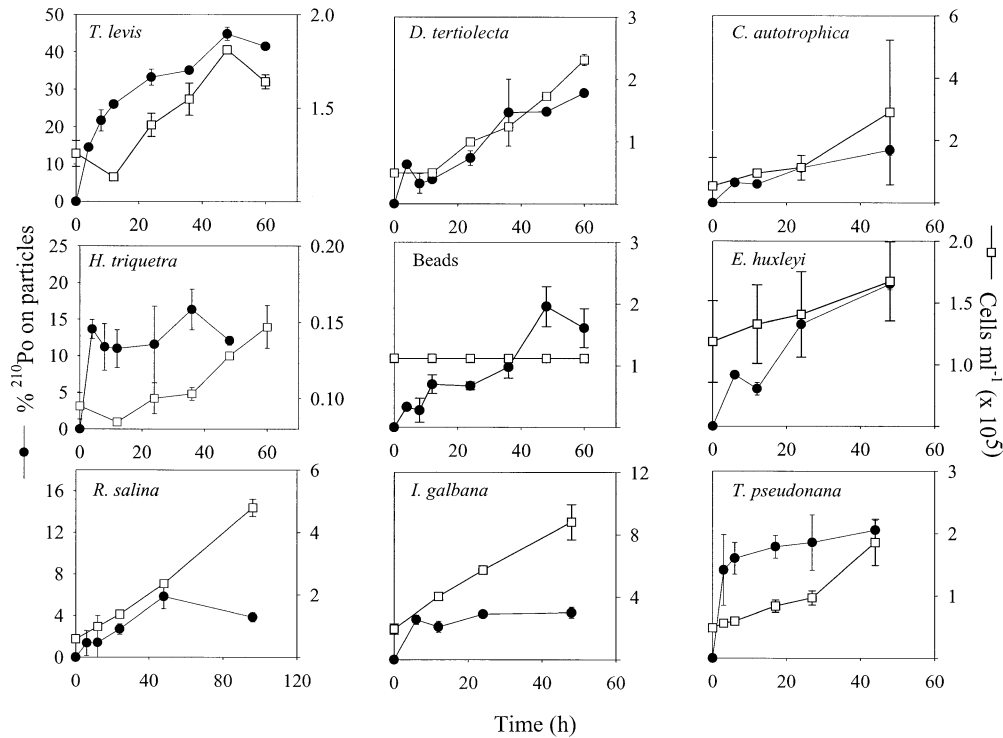


Fig. 1. Growth of eight species of marine phytoplankton and uptake of ^{210}Po by these cells and by glass beads over time. The left axis represents the percentage of total ^{210}Po in the $>1\text{-}\mu\text{m}$ fraction, the right axis represents the number of cells or beads ($\text{ml}^{-1} \times 10^5$). Data points are means from three replicate cultures and are shown with 1 SD error bars. Note the different scales on each graph.

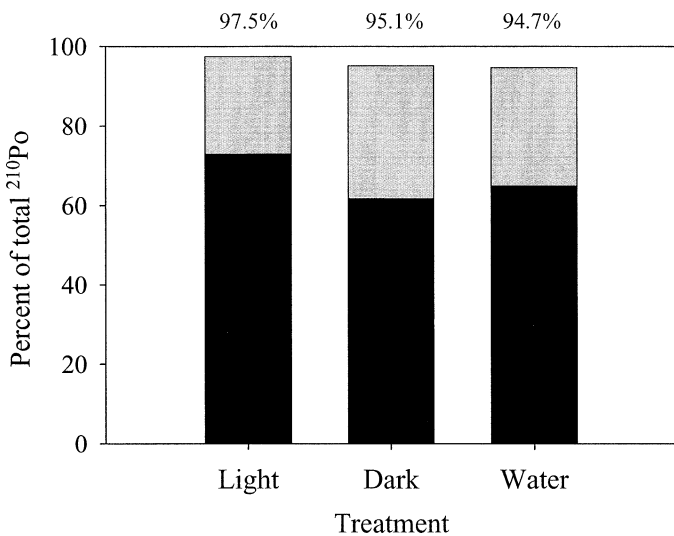


Fig. 2. Distribution of ^{210}Po at the end of uptake experiments in *T. pseudonana* cultures, maintained in constant light or constant dark, and in filtered seawater (14:10 LD). The black bars represent the percentage of total ^{210}Po in the final suspension. The gray bars represent the percentage of total ^{210}Po recovered from the flask walls with acid rinses. Each bar represents the mean of duplicate experiments. Total recoveries (%) of the initial ^{210}Po added to the flasks are also shown.

from the medium (Fig. 1). *T. levis*, *D. tertiolecta*, and *C. autotrophica* removed between 20 and 50% of the dissolved ^{210}Po from solution. *H. triquetra*, the glass beads, and *E. huxleyi*, despite their large differences in surface characteristics, scavenged between 10 and 20% of the metal from solution, whereas *R. salina*, *I. galbana*, and *T. pseudonana* accumulated between 4 and 12% of the total ^{210}Po in the culture flask. These differences in the amount of ^{210}Po scavenged were largely due to differences in biomass, or suspended particulate surface area, in the flasks. Generally, the uptake of ^{210}Po by the particles was initially very rapid and slowed considerably during the experiment, particularly for those cultures in which biomass leveled off over time. No detectable ^{210}Po was lost to volatilization during the course of the experiments, but substantial loss of the isotope to the glass walls was observed—from ~20 to 35% was recovered from acid rinses of the flask walls (Fig. 2).

Volume concentration factors (VCFs, $\text{Bq } ^{210}\text{Po } \mu\text{m}^{-3} \text{ cell} / \text{Bq } ^{210}\text{Po } \mu\text{m}^{-3}$ in the dissolved phase [$<0.2 \mu\text{m}$]) (Fisher et al. 1983a) were calculated during the ^{210}Po uptake period (Fig. 3). VCFs were calculated at each sample time. During the filtration process, some ^{210}Po sorbed to the filters; the activity on control filters, only exposed to dissolved polonium in sterile-filtered seawater, was very low and was subtracted from all particulate ^{210}Po values. VCFs for ^{210}Po ranged between 5×10^3 and 3×10^4 for the species tested. The mean VCF for each species and the glass beads was taken as the average calculated value for all time points after

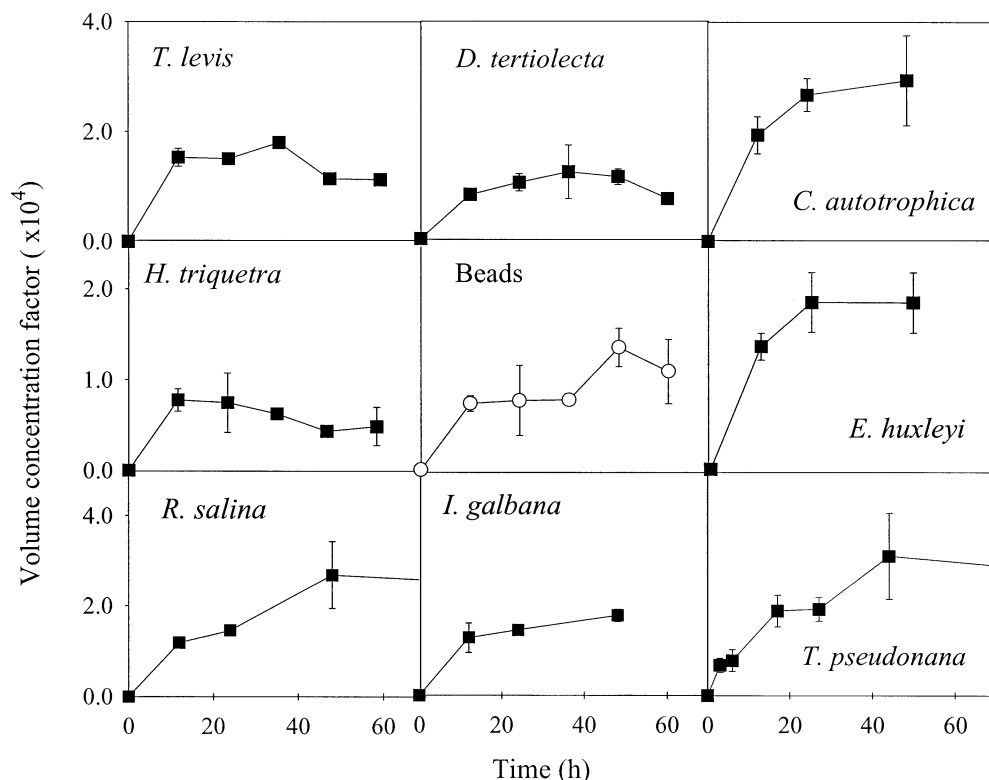


Fig. 3. ^{210}Po volume concentration factors (VCFs) for the eight algal species and the glass beads over time. Data points are means from three replicate cultures and are shown with 1 SD error bars.

12 h because this was the length of time after which there was little change in concentration factor.

Tests to assess the effects of EDTA and polonium concentration (up to 10 nmol L^{-1}) on ^{210}Po uptake and cellular growth by the diatom *T. pseudonana* showed no substantial effect of these variables (data not shown). In experiments to evaluate the effects of light and temperature on ^{210}Po uptake by diatoms, no significant effect of either condition was observed (Fig. 4).

For the ^{210}Po loss experiments, the cell density of the resuspended cells was the same as at the end of the ^{210}Po exposure period. Thus, if the distribution of ^{210}Po between cells and the dissolved phase was simply governed by equi-

librium partitioning, the fraction of the total ^{210}Po in the algal cell suspensions remaining bound to the cells at the end of the loss period should equal the fraction bound to cells at the end of ^{210}Po uptake. In fact, the cells did not lose as much ^{210}Po as would be predicted by equilibrium partitioning. The resuspended cells lost between 10 and 50% of their ^{210}Po into the dissolved phase, most within the first 24 h of the “depuration period,” after which loss rates were very low (Fig. 5). In contrast, the glass beads lost almost 85% of the ^{210}Po sorbed to their surface, in agreement with equilibrium expectations.

Discussion

All species of phytoplankton and the glass beads accumulated ^{210}Po from the dissolved phase; uptake was generally complete after 24 h. Because uptake of ^{210}Po was not affected by light or temperature, it appears that passive adsorption to cell surfaces controls the uptake of this element, as shown for many other metals (Fisher and Went 1993; Fisher and Reinfelder 1995).

Our concentration factors (VCFs) agreed well with the few studies that have investigated phytoplankton uptake of ^{210}Po . Heyraud and Cherry (1979) reported a ^{210}Po wet weight concentration factor of 0.9×10^4 for generic phytoplankton in seawater, and our mean VCF for all species was 1.5×10^4 . Our ^{210}Po VCFs for *T. pseudonana* and *D. tertiolecta* are lower than those found by Fisher et al. (1983b) for the same organisms. In fact, our VCFs for these

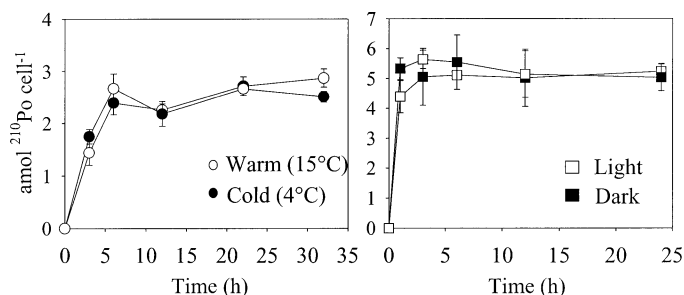


Fig. 4. ^{210}Po uptake by *T. pseudonana* grown at 4 and 15°C (14:10 LD) exposed to 5 nmol L^{-1} ^{210}Po and under constant light and constant dark (15°C) exposed to 10 nmol L^{-1} . Data points are means of three replicate cultures and are shown with 1 SD error bars.

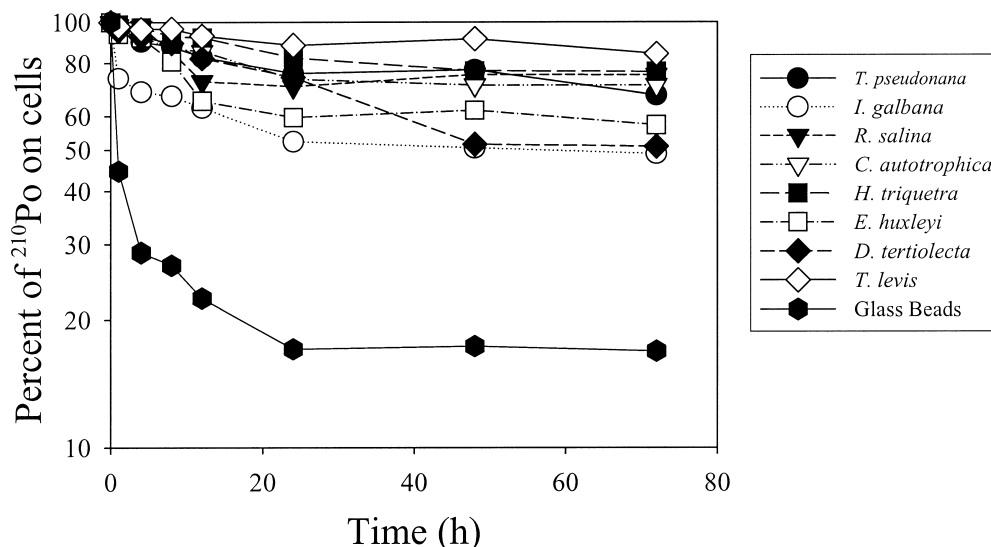


Fig. 5. Loss of ^{210}Po from radiolabeled phytoplankton and glass beads over 3 d. Each line represents the mean of duplicate experiments. At the beginning of the depuration period, 100% of the ^{210}Po was particulate.

species were four to six times lower than those found in the previous study (*T. pseudonana*: 2×10^4 compared to 12×10^4 ; *D. tertiolecta*: 1×10^4 compared to 4×10^4). The discrepancy between our VCFs and this earlier study could be due to the very different ^{210}Po concentrations used in the experiments. We used about $7 \times 10^4 \text{ Bq L}^{-1}$, whereas Fisher et al. (1983b) used 37 Bq L^{-1} , a concentration that is still four to five orders of magnitude higher than concentrations in natural waters ($0.3\text{--}3 \text{ mBq L}^{-1}$, Hong et al. 1999). Although we used three orders of magnitude more ^{210}Po than Fisher et al. (1983b), our VCFs only differed by a factor of four to six and compare well with the concentration factors calculated for phytoplankton measured in the field (Heyraud and Cherry 1979).

Another difference in experimental protocols from earlier laboratory experiments could also account for different ^{210}Po VCFs. Unlike in the earlier work in which ^{210}Po was added directly to algal suspensions, we let the ^{210}Po equilibrate for 24 h in filtered seawater before adding the cells or glass beads because we found that ^{210}Po was more bioavailable in the first 12 h after addition to seawater (data not shown). This change in the bioavailability of ^{210}Po over time was presumably due to changes in its speciation, perhaps reflecting the time necessary for the ^{210}Po to be complexed with dissolved organic compounds, which might reduce the sorption of this metal to the particles. Based on these preliminary experiments, Po VCFs in phytoplankton determined without first equilibrating the ^{210}Po with ambient seawater could have been up to an order of magnitude greater than the values shown in Table 1.

Despite obvious differences in cell surface characteristics between different algal taxa and the glass beads, all particles had similar VCFs for ^{210}Po , although species-specific variability was noted. The concentration factors of ^{210}Po in the particles studied followed the trend: *C. autotrophica* > *T. pseudonana* > *E. huxleyi* = *R. salina* = *I. galbana* > *T.*

levis > *D. tertiolecta* = glass beads > *H. triquetra*. The VCF range for phytoplankton cells measured here, $0.5\text{--}3 \times 10^4$, is lower than that reported for Th, about the same as Pb, and higher than U and Ra, among other natural series radionuclides (Fisher et al. 1983b, 1987). VCFs for other metals in phytoplankton also exceed that of ^{210}Po in some cases (e.g., Am, Pu, Ag, Hg) and are below ^{210}Po for other metals (e.g., Cd, Co, Mn) (Fisher 1986).

In an effort to better interpret our results, we created a simple model to explain interspecific variability in the cellular polonium content of algal cells. The model differentiates between ^{210}Po adsorbed to the external surface of the cell and ^{210}Po associated with cellular proteins. These two pools were considered because ^{210}Po is reactive for particle surfaces and is known to associate with sulfur in single-celled organisms (Fisher et al. 1983a; Cherrier et al. 1995; LaRock et al. 1996). Because the partitioning of ^{210}Po between the cells and dissolved phase seemed to reach an equilibrium during the uptake experiments (Fig. 1), we presume that the ^{210}Po content of the cells is in equilibrium with the ambient aqueous ^{210}Po pool; for the model, it is also assumed that the two cellular pools of ^{210}Po are in equilibrium with each other.

Based on these assumptions, cellular ^{210}Po , Po_c , can be expressed as a simple function of ambient aqueous ^{210}Po concentration, $[\text{Po}_w]$, cellular surface area, SA_c , and cell protein content, P_c , according to

$$\text{Po}_c = [\text{Po}_w] \{ K_{sa} \times \text{SA}_c + K_p \times \text{P}_c \} \quad (1)$$

where K_{sa} is the ^{210}Po partition coefficient for the surface-adsorbed pool ($\text{Po}_c / \{ [\text{Po}_w] \times \text{SA}_c \}$), and K_p is the partition coefficient for the protein-bound ^{210}Po pool ($\text{Po}_c / \{ [\text{Po}_w] \times \text{P}_c \}$). By dividing both sides of the equation by $[\text{Po}_w]$ and cell volume (V_c), we can solve algebraically for the ^{210}Po VCF (VCF_{Po}).

$$\text{VCF}_{\text{Po}} = (K_{\text{sa}} \times \text{SA}/V_c) + (K_p \times P_c/V_c) \quad (2)$$

Equation 2 indicates that VCF_{Po} should be predictable using a simple linear bivariate regression on SA/V_c and P_c/V_c .

In order to create the model, we began by examining the relationship between the size of cells and ^{210}Po VCF. Figure 6A shows the regression between mean SA/V and mean VCF for all eight species and the glass beads ($r^2 = 0.57$). Like ^{210}Po , the VCFs of other particle-reactive, nonessential metals are also positively correlated with cell surface area: volume ratios (Fisher and Reinfeldter 1995).

We next examined the relationship between VCF and cellular protein content. By plotting the residuals from Fig. 6A against mean protein content of the particles (Fig. 6B), we could account for much of the variation from the regression line in Fig. 6A ($r^2 = 0.67$). Cells with more protein had more total ^{210}Po associated with them than would be predicted based on their size alone. From these results, we created a linear bivariate model in which SA/V_c and $\text{protein}/V_c$ are used to explain 85% of the variance in VCFs across taxa (Fig. 6C). The coefficients for both variables were highly significant. The model equation was

$$\text{VCF}_{\text{Po}} = 12,335 \times \text{SA}/V_c + 76,815 \times P_c/V_c \quad (3)$$

with standard errors of $\pm 2,634$ for K_{sa} and $\pm 2,647$ for K_p . Both coefficients are positive, indicating that smaller cells (higher SA/Vol) with high protein content will have the highest concentration of ^{210}Po .

Adding other measured parameters to the model, such as cell wall material and C:N ratios, did not improve the relationship between model-predicted and observed VCFs (data not shown). Because it appears that VCF_{Po} depends largely on size and protein content, characteristics such as community size structure or nutrient status could influence both the amount of ^{210}Po associated with cells and the distribution of the isotope within the cells themselves. This partitioning would then affect both trophic transfer of ^{210}Po (Reinfeldter and Fisher 1991) and the usefulness of ^{210}Po as a tracer for organic matter (Friedrich and Rutgers van der Loeff 2002).

As with earlier studies (Fisher et al. 1983b; Cherrier et al. 1995) and as our bivariate model assumed, the ^{210}Po in our experiments was not only associated with the cell surfaces but penetrated to varying degrees (30–60% of total cellular ^{210}Po) into the cytoplasm of the different algal species. Furthermore, the extent to which the ^{210}Po was distributed in algal cytoplasm was comparable to that in the Fisher et al. (1983b) study, in which much lower ^{210}Po concentrations were used experimentally. For example, we found that about 40% of the ^{210}Po associated with *T. pseudonana* was within the cytoplasm, whereas Fisher et al. found 38% inside the cytoplasm for this species. However, in *D. tertiolecta*, our cytoplasmic ^{210}Po comprised almost 60% of the total cellular ^{210}Po , whereas Fisher et al. (1983b) found only 31% in the cytoplasm of this species. This discrepancy might result from the higher dissolved ^{210}Po concentrations in our cultures, which possibly overwhelmed the capacity of the membranes of this naked flagellate to bind the ^{210}Po . It is noteworthy that ^{210}Po penetrates to a much greater degree into algal cytoplasm than does Pb (Fisher et al. 1983b) and other

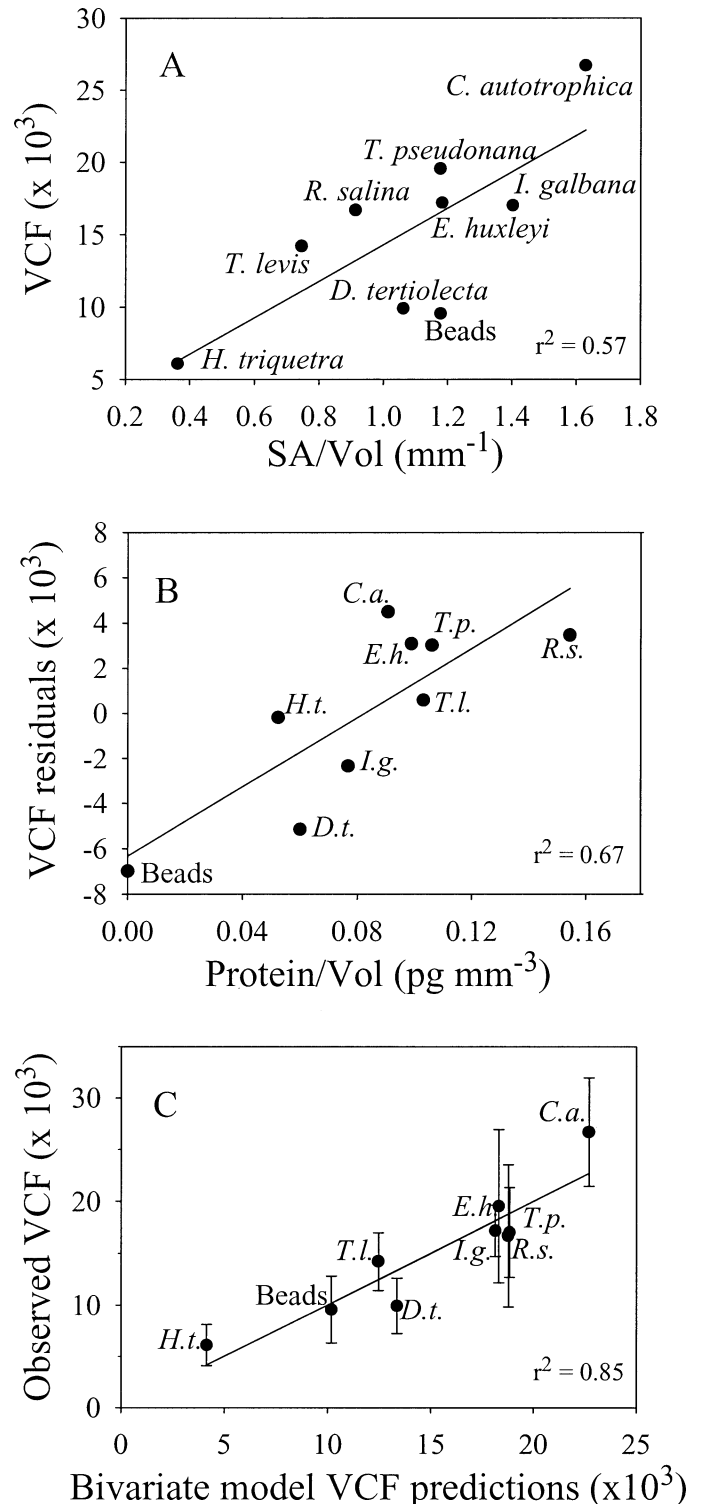


Fig. 6. (A) Correlation between the surface area to volume ratio of particles and volume concentration factor (VCF, $\times 10^3$). Points represent mean SA/V ratios and mean VCFs ($n = 3$). (B) The residuals (distance from the regression line) from panel A versus the protein content normalized to volume for the particles. Note the point at zero protein is the glass beads. (C) The observed VCFs in this experiment compared to predictions of the bivariate model. Data points are means from three replicate cultures and are shown with 1 SD error bars. The solid line is the 1:1 line.

particle-reactive nonessential class A metals like Am and class B metals like Ag and Hg (Reinfelder and Fisher 1991; Mason et al. 1996). In fact, even essential metals like Zn, Fe, and Cu are less enriched than ^{210}Po in the cytoplasm of these species (Reinfelder and Fisher 1991; Hutchins et al. 1995; Chang and Reinfelder 2000).

The mechanism by which the ^{210}Po enters the cytoplasm of these cells remains unstudied. Cherrier et al. (1995) followed uptake kinetics of Po and ^{35}S into bacteria cells and found that the patterns of uptake were different but the final distribution within the cells was similar for these two elements. Fisher et al. (1983b) also found ^{210}Po associated with the S-containing protein fraction of two species of phytoplankton cells.

The loss of ^{210}Po from phytoplankton cells could not be predicted using equilibrium adsorption partitioning (Fig. 5), whereas the loss from the glass beads was predictable. This result again suggests that, unlike the case of glass beads, there could be two or more pools of ^{210}Po associated with phytoplankton cells: one that is reversibly bound to the exterior (the predictable pool on the beads) and one that is bound to internal cell constituents or covalently bound to protein on cell surfaces. In contrast to Po, the partitioning of Th and Pb between the dissolved phase and particulate matter is predictable using reversible adsorption/desorption processes (Bacon and Anderson 1982; Fisher et al. 1987), which could reflect an association of these metals with only the surface of particles.

We calculated the biological half-life ($t_{b,1/2}$) of ^{210}Po using the mean loss rate from the slowly exchanging pool for all species, assuming the rapidly exchanging ^{210}Po on the cells (presumably surface-bound) has already reached equilibrium (within 24 h). On the basis of our results, the cells would lose half of their ^{210}Po in approximately 35 d when maintained without grazing at 4°C , not accounting for radioactive decay. Taking the 138-d radioactive half-life of ^{210}Po into account, the time to lose half the ^{210}Po on cells would be reduced to 23 d. This $t_{b,1/2}$ is much shorter than those previously recorded for Ag, Am, Cd, Ce, Co, Pb, and Se at similar temperatures but comparable to protein in dying phytoplankton cells (25 d) (Lee and Fisher 1993). However, this half-life is relatively long compared to the turnover rate of plankton and their carbon in surface waters (Lee and Fisher 1993; Reinfelder et al. 1993). Because much of the ^{210}Po was incorporated into the cytoplasm of phytoplankton cells and associated with protein, much of the ^{210}Po bound to phytoplankton cells would likely be assimilated by zooplankton feeding on these cells (Reinfelder and Fisher 1991). This transfer from one trophic level to another would be expected to generate longer residence times for ^{210}Po within the surface ocean food web.

This work could be a first step toward understanding the bioconcentration of ^{210}Po in marine plankton with an eye toward utilizing it as a chemical tracer for the flux of particulate organic matter in the surface ocean. Potentially, the amount of ^{210}Po associated with phytoplankton, regardless of the taxa present, in the ocean's surface can be predicted using only two basic cell characteristics: size and protein content. This has far-reaching implications both in terms of the trophic transfer and bioconcentration of polonium, as well

as augmenting its usefulness as a tracer. The results of our experiments, including the predictive power of our bivariate model; the evidence of penetration of ^{210}Po into algal cytoplasm; and the long half-life of the intracellular ^{210}Po suggest that attempts at using ^{210}Po as a chemical tracer of organic matter (Friedrich and Rutgers van der Loeff 2002) are likely to be successful.

References

- BACON, M. P., AND R. F. ANDERSON. 1982. Distribution of thorium isotopes between dissolved and particulate forms in the deep sea. *J. Geophys. Res.* **46**: 1293–1299.
- , D. W. SPENCER, AND P. G. BREWER. 1976. $^{210}\text{Pb}/^{226}\text{Ra}$ and $^{210}\text{Po}/^{210}\text{Pb}$ disequilibria in seawater and suspended particulate matter. *Earth Planet. Sci. Lett.* **32**: 277–296.
- BULMAN, R. A., L. W. EWERS, AND K. MATSUMOTO. 1995. Investigation of the potential bioavailability of ^{210}Po in some food-stuffs. *Sci. Total Environ.* **173/174**: 151–158.
- CARVALHO, F. P. 1997. Distribution, cycling and mean residence time of ^{226}Ra , ^{210}Pb and ^{210}Po in the Targus estuary. *Sci. Total Environ.* **196**: 151–161.
- CHANG, S. I., AND J. R. REINFELDER. 2000. Bioaccumulation, sub-cellular distribution, and trophic transfer of copper in a coastal marine diatom. *Environ. Sci. Technol.* **34**: 1084–1101.
- CHERRIER, J., W. C. BURNETT, AND P. A. LAROCK. 1995. Uptake of polonium and sulfur by bacteria. *Geomicrobiol. J.* **13**: 103–115.
- CHERRY, R. D. 1964. Alpha-radioactivity of plankton. *Nature* **203**: 139–143.
- , AND M. HEYRAUD. 1981. Polonium-210 content of marine shrimp: Variation with biological and environmental factors. *Mar. Biol.* **65**: 165–175.
- CLULOW, F. V., N. K. DAVE, T. P. LIM, AND R. AVADHANULA. 1998. Radionuclides (lead-210, polonium-210, thorium-230, and -232) and thorium and uranium in water, sediments, and fish from lakes near the city of Elliot Lake, Ontario, Canada. *Environ. Pollut.* **99**: 199–213.
- COCHRAN, J. K., M. P. BACON, S. KRISHNASWAMI, AND K. K. TURKIAN. 1983. ^{210}Po and ^{210}Pb distributions in the central and eastern Indian Ocean. *Earth Planet. Sci. Lett.* **65**: 433–452.
- DAHLGAARD, H. 1996. Polonium-210 in mussels and fish from the Baltic-North Sea Estuary. *J. Environ. Radioact.* **32**: 91–96.
- DURAND, J. P., F. P. CARVALHO, F. GOUDARD, J. PIERI, S. W. FOWLER, AND O. COTRET. 1999. ^{210}Po binding to metallothioneins and ferritin in the liver of teleost marine fish. *Mar. Ecol. Prog. Ser.* **177**: 189–196.
- FISHER, N. S. 1986. On the reactivity of metals for marine phytoplankton. *Limnol. Oceanogr.* **31**: 443–449.
- , AND J. R. REINFELDER. 1995. The trophic transfer of metals in marine systems, p. 363–406. *In* A. Tessier and D. R. Turner [eds.], *Metal speciation and bioavailability in aquatic systems*. Wiley.
- , AND M. WENTE. 1993. The release of trace elements by dying marine phytoplankton. *Deep-Sea Res.* **40**: 671–694.
- , P. BJERREGAARD, AND S. W. FOWLER. 1983a. Interactions of marine plankton with transuranic elements. I. Biokinetics of neptunium, plutonium, americium, and californium in phytoplankton. *Limnol. Oceanogr.* **28**: 432–447.
- , K. A. BURNS, R. D. CHERRY, AND M. HEYRAUD. 1983b. Accumulation and cellular distribution of ^{241}Am , ^{210}Po , and ^{210}Pb in two marine algae. *Mar. Ecol. Prog. Ser.* **11**: 233–237.
- , J.-L. TEYSSIE, S. KRISHNASWAMI, AND M. BASKARAN. 1987. Accumulation of Th, Pb, U, and Ra in marine phyto-

- plankton and its geochemical significance. *Limnol. Oceanogr.* **32**: 131–142.
- , R. R. L. GUILLARD, AND D. C. BANKSTON. 1991. The accumulation of barium by marine phytoplankton grown in culture. *J. Mar. Res.* **49**: 339–354.
- FRIEDRICH, J., AND M. M. RUTGERS VAN DER LOEFF. 2002. A two tracer (^{210}Po – ^{234}Th) approach to distinguish organic carbon and biogenic silica export flux in the Antarctic Circumpolar Current. *Deep-Sea Res.* **49**: 101–120.
- GERMAIN, P., G. LECLERC, AND S. SIMON. 1995. Transfer of polonium-210 into *Mytilus edulis* (L.) and *Fucus vesiculosus* (L.) from the Baie de Seine (Channel coast of France). *Sci. Total Environ.* **164**: 109–123.
- GUILLARD, R. R. L., AND J. H. RYTHER. 1962. Studies of marine planktonic diatoms I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.* **8**: 229–239.
- HEYRAUD, M., AND R. D. CHERRY. 1979. Polonium-210 and lead-210 in marine food chains. *Mar. Biol.* **52**: 227–236.
- HONG G.-H., S.-K. PARK, M. BASKARAN, S.-H. KIM, C.-S. CHUNG, AND S.-H. LEE. 1999. Lead-210 and polonium-210 in the winter well-mixed turbid waters in the mouth of the Yellow Sea. *Cont. Shelf Res.* **19**: 1049–1064.
- HUTCHINS, D. A., W.-X. WANG, AND N. S. FISHER. 1995. Copepod grazing and the biogeochemical fate of diatom iron. *Limnol. Oceanogr.* **40**: 989–994.
- KADKO, D., M. P. BACON, AND A. HUDSON. 1987. Enhanced scavenging of ^{210}Pb and ^{210}Po by processes associated with the East Pacific Rise near 8°45'N. *Earth Planet. Sci. Lett.* **81**: 349–357.
- LAROCK, P., J.-H. HYUN, W. C. BURNETT, AND C. D. HULL. 1996. Bacterial mobilization of polonium. *Geochim. Cosmochim. Acta* **60**: 4321–4328.
- LEE, B.-G., AND N. S. FISHER. 1993. Release rates of trace elements and protein from decomposing planktonic debris. 1. Phytoplankton debris. *J. Mar. Res.* **51**: 391–421.
- MASON, R. P., J. R. REINFELDER, AND F. M. M. MOREL. 1996. Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. *Environ. Sci. Technol.* **30**: 1835–1845.
- MOMOSHIMA, N., L.-X. SONG, S. OSAKI, AND Y. MAEDA. 2001. Formation and emission of volatile polonium compounds by microbial activity and polonium methylation with methylcobalamin. *Environ. Sci. Technol.* **35**: 2956–2960.
- MOORE, R. M., AND J. N. SMITH. 1986. Disequilibria between ^{226}Ra , ^{210}Pb and ^{210}Po in the Arctic Ocean and the implications for chemical modification of the Pacific water inflow. *Earth Planet. Sci. Lett.* **77**: 285–292.
- NOZAKI, Y., J. ZHANG, AND A. TAKEDA. 1997. ^{210}Pb and ^{210}Po in the equatorial Pacific and the Bering Sea: The effects of biological productivity and boundary scavenging. *Deep-Sea Res.* **44**: 2203–2220.
- , F. DOBASHI, Y. KATO, AND Y. YAMAMOTO. 1998. Distribution of Ra isotopes and the ^{210}Pb and ^{210}Po balance in surface seawaters of the mid Northern Hemisphere. *Deep-Sea Res.* **45**: 1263–1284.
- PENTREATH, R. J., AND D. J. ALLINGTON. 1988. Dose to man from the consumption of marine seafoods: A comparison of the naturally occurring ^{210}Po with artificially-produced radionuclides, p. 1582–1585. *In Proceedings of the 7th International Congress of the I.R.P.A.*, Sydney.
- RADAKOVITCH, O., R. D. CHERRY, AND S. HEUSSNER. 1999. ^{210}Pb and ^{210}Po : Tracers of particle transfer on the Rhone continental margin (NW Mediterranean). *Deep-Sea Res.* **46**: 1539–1563.
- REINFELDER, J. R., AND N. S. FISHER. 1991. The assimilation of elements ingested by marine copepods. *Science* **251**: 794–796.
- , S. W. FOWLER, AND J.-L. TEYSSIE. 1993. Release rates of trace elements and protein from decomposing planktonic debris. 2. Copepod carcasses and sediment trap particulate matter. *J. Mar. Res.* **51**: 423–442.
- RUBIN, K. 1997. Degassing of metals and metalloids from erupting seamount and mid-ocean ridge volcanoes: Observations and predictions. *Geochim. Cosmochim. Acta* **61**: 3525–3542.
- SHANNON, L. V., R. D. CHERRY, AND M. J. ORREN. 1970. Polonium-210 and lead-210 in the marine environment. *Geochim. Cosmochim. Acta* **34**: 701–711.
- SHEELER, P. 1981. *Centrifugation in biology and medical science*. Wiley.
- SMITH, P. K., AND OTHERS. 1985. Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150**: 76–85.
- STEPNOWSKI, P., AND B. SKWARZEC. 2000. A comparison of ^{210}Po accumulation in molluscs from the southern Baltic, the coast of Spitzbergen and Sasek Wielki Lake in Poland. *J. Environ. Radioact.* **49**: 201–208.

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