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COMPARISON OF SELENOMETHIONINE AND SELENITE CYCLING IN FRESHWATER EXPERIMENTAL PONDS

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Abstract. A comparison of the cycling and fate of ^{75}Se in an aquatic ecosystem was investigated when selenite or selenomethionine was introduced as an acute release into two experimental ponds. Biotic and abiotic compartments were periodically sampled to measure ^{75}Se concentrations throughout the 318-d experiment. The biotic compartments were periphyton, rooted pondweed (*Elodea canadensis*), snails (*Helisoma* sp.), and mosquitofish (*Gambusia affinis*). Results show that the uptake rates in the biotic compartments for ^{75}Se-selenomethionine were at least an order of magnitude greater than for ^{75}Se-selenite. However, elimination rates for respective biotic compartments were similar. Seasonal sediment ^{75}Se concentrations in both ponds decreased simultaneously with increases in ^{75}Se concentrations within biotic compartments. This suggests that sediment is a secondary source of ^{75}Se rather than a sink in some shallow freshwater systems.

1. Introduction

Selenium may exist in three dissolved oxidation states in freshwater systems; dissolved organic selenides, selenite, and selenate (Cutter, 1986). Selenite has been shown to be the predominant Se species in many power plant cooling reservoirs and in several Eastern United States reservoirs and lakes (Cutter, 1989). However, in natural waters dissolved organic compounds, which may be produced from oxidation-reductions and biological processes (Shrift, 1973; Chau *et al.*, 1976), are the predominant selenide species (Cutter, 1982; 1986). Selenomethionine (SeMet) was isolated and identified as one major dissolved organic selenide constituent (Ganther and Hsieh, 1974; Doran and Alexander, 1976).

Food chain and microcosm studies (Hesslein *et al.*, 1980; Turner and Rudd, 1983; Besser *et al.*, 1989) have shown that the rate of Se uptake by biota in freshwater systems is dependent upon the chemical forms of Se. Algae, macrophytes, and other primary producers are able to remove selenite directly from the water column but may preferentially take up dissolved organic forms of Se, such as selenomethionine (Sandholm *et al.*, 1973). Primary producers can metabolically reduce inorganic Se, such as selenite, to organic forms (selenoamino acids) which are more efficiently assismilated by higher trophic level organisms (Bottino *et al.*, 1984). The

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uptake, concentration, transfer and depuration kinetics varies with each Se species in aquatic biota and may contribute to the observed toxic effects (organ necrosis, reproductive failure, and mortality) which have been noted (Sorensen *et al.*, 1984; Presser and Barnes, 1984; Garrett and Inman, 1984; Saiki, 1985). Most laboratory Se toxicity experiments have concentrated on acute exposures whereas the studies of natural systems have concentrated on chronic exposures of aquatic biota in Se contaminated freshwater lakes and reservoirs after toxicity has already been observed (Bertram and Brooks, 1986; Cumbie, 1978). Few studies have focused on the uptake and elimination of Se within natural systems comparing various Se species, aquatic biota, and waterborne transfer routes.

The objectives of this study were to elucidate and quantify selenomethionine and selenite: (i) uptake within each biotic and abiotic pond compartment; (ii) transfer from the abiotic compartments into and through the biotic compartments; and (iii) elimination and depuration rates of Se in biotic compartments utilizing radioactive ^{75}Se as a tracer.

2. Materials and Methods

The two experimental ponds and a reference pond used in this study had an average volume of 6.2 m^3 and were lined with a inert plastic covering which prevented water leakage. The liner bottom was covered with 15 cm of sediment. Further pond preparations are described in other publications (Blaylock *et al.*, 1982; Giddings *et al.*, 1984). Pondweed, (*Elodea canadensis*) weighing 4.8 kg (drained wet wt), 150 individually-marked snails (*Helisoma* sp.), 250 mosquitofish (*Gambusia affinis*), and a natural assemblage of pond microflora and fauna (zooplankton, algae, fungi, bacteria, and insects) were collected locally and added to the ponds. The biota was allowed to develop for 3 mo prior to the introduction of the ^{75}Se compounds. Representative samples were analyzed to ensure that each biotic compartment had not been previously exposed to ^{75}Se. The water quality parameters (pH, temperature, conductivity, alkalinity, and dissolved oxygen) of each pond were monitored throughout the 318-d experiment with the ponds' parameters varying $\leq 10\%$.

Selenomethionine (^{75}Se-SeMet) and ^{75}Se-Selenite (Specific Activity of each species was 740 MBq mg^{-1}) were purchased from Amersham Corp., U.S.A. Three hundred mL of ^{75}Se-SeMet (50 kBq mL^{-1}) and ^{75}Se-Selinite (66 kBq mL^{-1}) were added into the respective experimental pond. While pouring ^{75}Se, the waters were stirred to disperse and distribute the ^{75}Se throughout the ponds. Biotic and abiotic samples were collected from each pond at 1 and 4 hr post-spike and then once per day for the first 5 d. Additional samples were collected as necessary throughout the course of the 318-d study. Sampling procedures were similar to those described by Blaylock and Frank (1982) and Giddings *et al.* (1984).

Five-hundred mL water samples were collected from each quadrant of the ponds using an integrated water column sampler. The samples were analyzed for ^{75}Se on a NaI(Tl) detector connected to a Nuclear Data 6700 multichannel analyzer

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decreasing. This observation would suggest that ^{75}Se is not only taken up directly from the water column but that a more significant chronic route contributing to ^{75}Se concentration in *Helisoma* sp. and *Gambusia affinis* would come from foodchain exposure (Cumbie and Van Horn, 1978; Lemly, 1985; Hamiliton *et al.*, 1986; Saiki and Lowe, 1987).

The elimination data (Figure 3) and biological half-lives (Table II) for ^{75}Se in this study are greater than those reported by Sato *et al.* (1980), Hodson *et al.* (1980), and Bertram and Brooks (1986) but similar to those found in Kesterson Reservoir (Horne, personal communication, 1990). Possible reasons for these different results are: (i) the use of different fish species; and (ii) the time and duration of this environmentally exposed experiment compared with the much shorter laboratory studies; and (iii) the combined use of selenomethionine and selenite uptake from both the water column and the food chain routes in an outdoor small pond rather than in a laboratory aqaurium. The CR's and the CF's (Table I) agree favorably with concentration ratios reported for organisms found in Belew's Reservoir and Kesterson Reservation (Lemly, 1985; Saiki and Lowe, 1987).

In conclusion, the initial rapid uptake of ^{75}Se-SeMet in the biotic compartments occurred through direct uptake from the water column. However as ^{75}Se in the water column decreased, the concentration of ^{75}Se in the *Helisoma* sp. and *G. affinis* biotic compartment continued to increase indicating the importance which the food chain plays in chronic freshwater ^{75}Se cycling. The similarity of the elimination rates may indicate a convergence to a common metabolic Se pool within the biota (Kleinow and Brooks, 1986). Selenium-75 appears to be mobilized from sediment of both ponds as an organo-selenium compound. The sediment would therefore act as a secondary source for selenium in shallow aerobic ponds and lakes rather than a terminal storage site or sink.

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TABLE I

Experimental Ponds' Mass Balance

Compartement	^{75}Se-SeMet Pond (% initial 75Se released into pond water)	^{75}Se-Selenite Pond
(Day 1)		
Water	85	97
Sediment	0.4	0.2
Periphyton	4.0	2.0
E. canadensis	6.0	0.4
Helisoma	0.1	0.02
G. affinis	0.4	0.006
Total	95.9%	99.6%
(Day 318)		
Water	36.0	38.7
Sediment	23.0	17.6
Periphyton	5.0	7.0
E. canadensis	17.0	19.0
Helisoma	0.6	1.1
G. affinis	3.4	3.6
Total	85%	87%

TABLE II

SeMet and Selenite biological concentration ratios (biota concentration/water concentration) and time integrated bioconcentration factors

Biota	Concentration Ratio (L kg^{-1})		Time-integrated concentration factor (L kg^{-1})	
	SeMet	Selenite	SeMet	Selenite
Periphyton[a]				
Day 4	2.4E+04	1.6E+02		
Day 127	3.0E+04	9.6E+03		
Day 318	4.2E+03	2.5E+03	1.6E+04	2.8E+03
Eloda canadensis[a]				
Day 4	3.2E+03	5.1E+01		
Day 127	6.0E+03	3.8E+03		
Day 318	1.2E+03	2.1E+03	4.9E+03	1.0E+03
Helisoma sp.				
Day 4	3.4E+02	5.0E+00		
Day 127	6.8E+03	1.0E+03		
Day 318	1.1E+03	9.5E+02	2.0E+03	2.5E+02
Gambusia affinis				
Day 4	1.7E+02	6.0E−01		
Day 127	1.0E+04	8.7E+01		
Day 318	2.6E+03	2.3E+03	3.3E+03	2.7E+02

[a] Dry weight; Dry: wet ratios of 1: 13.8 and 1: 10.5 were calculated for *Periphyton* and *Elodea canadensis*, respectively.

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TABLE III

Biological halflives of Se in biota exposed to ^{75}Se-Selenomethionine and ^{75}Se-Selenite

	Selenomethionine	Selenite
Periphyton	29 ± 4.9 d	28 ± 3.2 d
Elodea canadensis	38 ± 3.1 d	35 ± 3.3 d
Helisoma sp.	87 ± 7.7 d	69 ± 6.8 d
Gambusia affinis	116 ± 15.6 d	77 ± 7.2 d

corresponding rapid biotic uptake and assimilation is in agreement with results previously reported (Hobbie *et al.*, 1968; Rudd *et al.*, 1980; Klaverkamp *et al.*, 1983; Turner and Rudd, 1983). The rapid uptake and assimilation of ^{75}Se-SeMet by *Helisoma* and *Gambusia* from the water may be due to the organic nature of the ^{75}Se compound, as methionine is an essential amino acid in snails, fish, and mammals (Hobbie *et al.*, 1968; Zubay, 1983). The slower and more gradual ^{75}Se-Selenite uptake was also previously observed by Rudd *et al.* (1980).

The sediment compartment in both ponds acted initially as a particulate sink for ^{75}Se leaving the water column. Even though ^{75}Se in the SeMet spiked pond had a greater affinity for particulates than the anionic selenite species, as evidenced by both filtered water and organic and/or cation particulate data, the sediment uptake between ponds was not statistically different (α = 0.05, t-test). Selenium-75 which was taken up by the biota was recycled to the water through fecal material, dead biota, and other organic debris comprising detrital rain as well as through sediment remobilization. Much of the detritus settles to the sediment as organic ^{75}Se which is readily dissolved and is in a bioavailable chemical from (Reamer and Zoller, 1980; Bottino *et al.*, 1984). The remobilization of ^{75}Se from sediment would explain the rapid increased concentration of ^{75}Se in water, *Helisoma* sp., and *G. affinis* compartments in both experimental ponds (commencing day 170, post-spike) and the two-fold increase of organic and/or cation fraction in the ^{75}Se-Selenite water column.

The *Periphyton* compartment rapid uptake of ^{75}Se-SeMet and ^{75}Se-Selenite is in agreement with earlier studies (Wrench, 1978; Turner and Rudd, 1983; Bottino *et al.*, 1984). Immediate uptake by macrophyte stem and leaves (Denny, 1972; Nassos *et al.*, 1980; Ausmus *et al.*, 1978) and chronic uptake through the roots (Cowgill, 1974) would explain the continuous ^{75}Se uptake in the rooted macrophytes throughout this experiment. The data suggests that rooted plants may act as an initial sink by storing and concentrating either ^{75}Se species. Then the aquatic macrophytes may act as a secondary ^{75}Se source both by releasing organic ^{75}Se (Bottino *et al.*, 1984) upon plant death and cell lysis and by acting as a food source to pond grazers.

Both ponds' biotic compartments reflected an increase in ^{75}Se compartmental concentration which continued even though the ^{75}Se water concentration was

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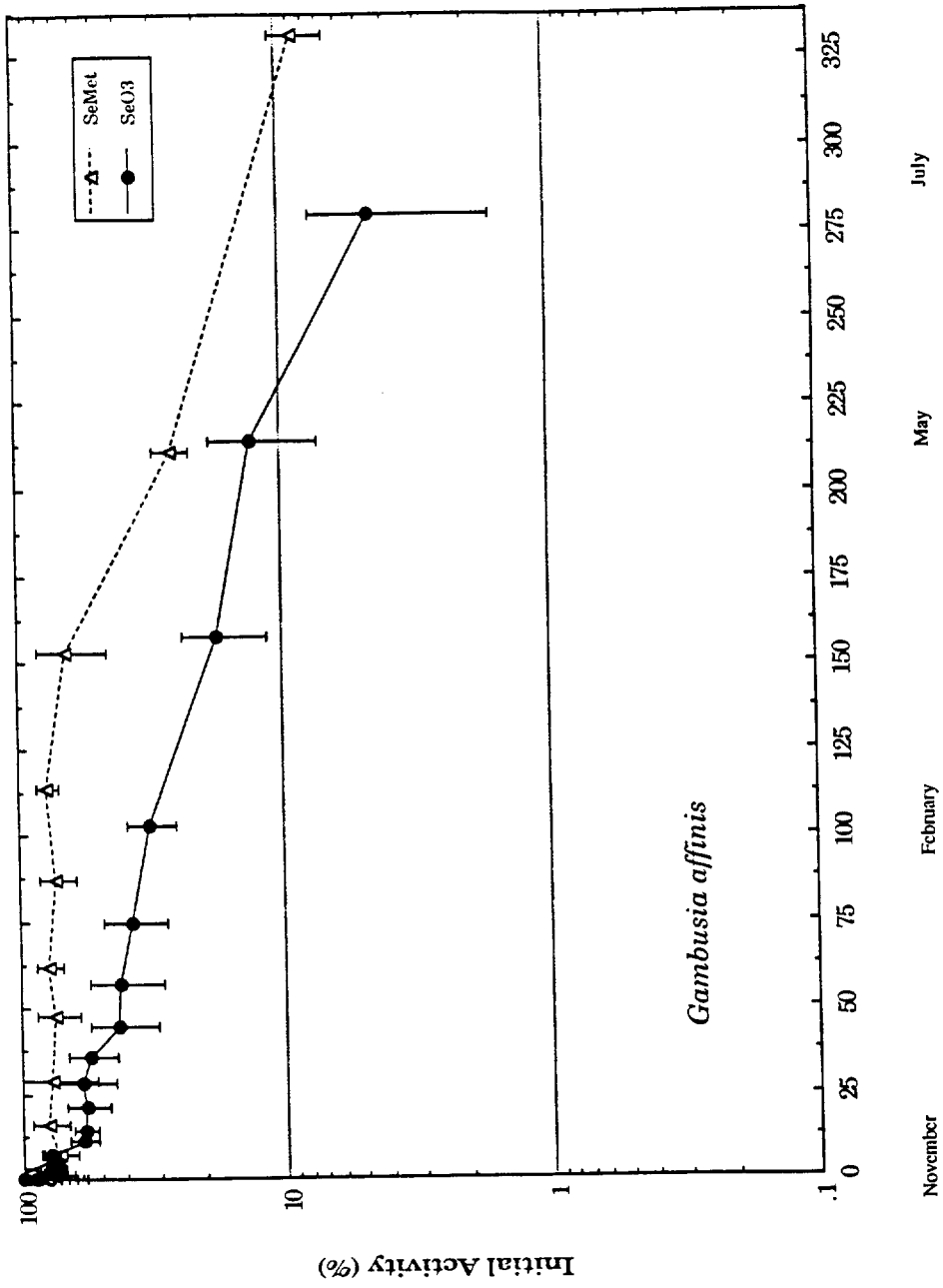

Fig. 3d.

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and 8.7×10^3 (day 318) for the ^{75}Se-SeMet and ^{75}Se-Selenite spiked pond, respectively. The sediment accumulated rapidly both ^{75}Se species as evidenced by the rapid transfer of ^{75}Se from the water column (Figure 1b). However, with seasonal warming ^{75}Se activity in the sediment decreased and the ^{75}Se activity in the water increased in both experimental ponds. This suggests that ^{75}Se is mobilized from the sediment. The results from the charcoal, copper-chelex, and cation-exchange columns showed that the organic and/or cation percentage in the water column of ^{75}Se-SeMet spiked pond ranged from 75% (day 27 post-spike) to 81% (day 318 post-spike) of the total activity; whereas in the ^{75}Se-Selenite spiked pond the organic and/or cation percentage ranging from 34% (day 27 post-spike) to 75% (day 318 post-spike) of the total activity. The experimental ponds' ^{75}Se mass balance is shown in Table I.

Selenium-75 activity in *Periphyton, E. canadensis, Helisoma* sp., and *G. affinis* increased rapidly during the first weeks in both experimental ponds (Figure 2) but was more rapidly taken up and concentrated by the biota in the ^{75}Se-SeMet spiked pond. Uptake of ^{75}Se by *Periphyton* was faster than other biotic pond compartments. The rapid uptake by all of the biotic compartments in the ^{75}Se-SeMet pond suggests initial direct uptake either through the stem and/or leaves for *E. canadensis* and via the gills and/or skin for *Helisoma* sp. and *G. affinis*. The concentrations within the ^{75}Se-Selenite exposed organisms gradually increased such that the concentration of ^{75}Se in corresponding experimental ponds' biotic compartments had converged by the end of the experiment and the concentration in all but the *Helisoma* sp. compartment was statistically equivalent ($\alpha = 0.05$, t-test).

In the elimination study, a two-phase elimination curve (Figure 3) was observed for all of the sampled biotic compartments for both species of ^{75}Se. The slopes of the long-term component of the elimination curve were nearly parallel indicating the similarity between the depuration rates of the two forms of Se.

Changes in the concentration ratios (CR) over time and the time-integrated concentration factors (CF) are shown for each pond biotic compartment in Table II. The rapid uptake of ^{75}Se-SeMet is reflected in the CF values which are approximately an order of magnitude greater than the ^{75}Se-Selenite CF values for each biotic compartment. The CF values also show that *Periphyton* and *E. canadensis* in both experimental ponds' biotic compartments more highly concentrate ^{75}Se than the other sampled biotic compartments.

The biological half-lives of ^{75}Se for *Periphyton, E. canadensis, Helisoma* sp., and *G. affinis* exposed to ^{75}Se-SeMet and ^{75}Se-Selenite are presented in Table III. The slopes of the lines shown in Figure 3 were used to determine the half-lives for the two Se species. Results indicate that the two forms of ^{75}Se are less than a factor of two different for each biotic compartment.

4. Discussion and Conclusions

The rapidity with which ^{75}Se-SeMet was removed from the water column and a

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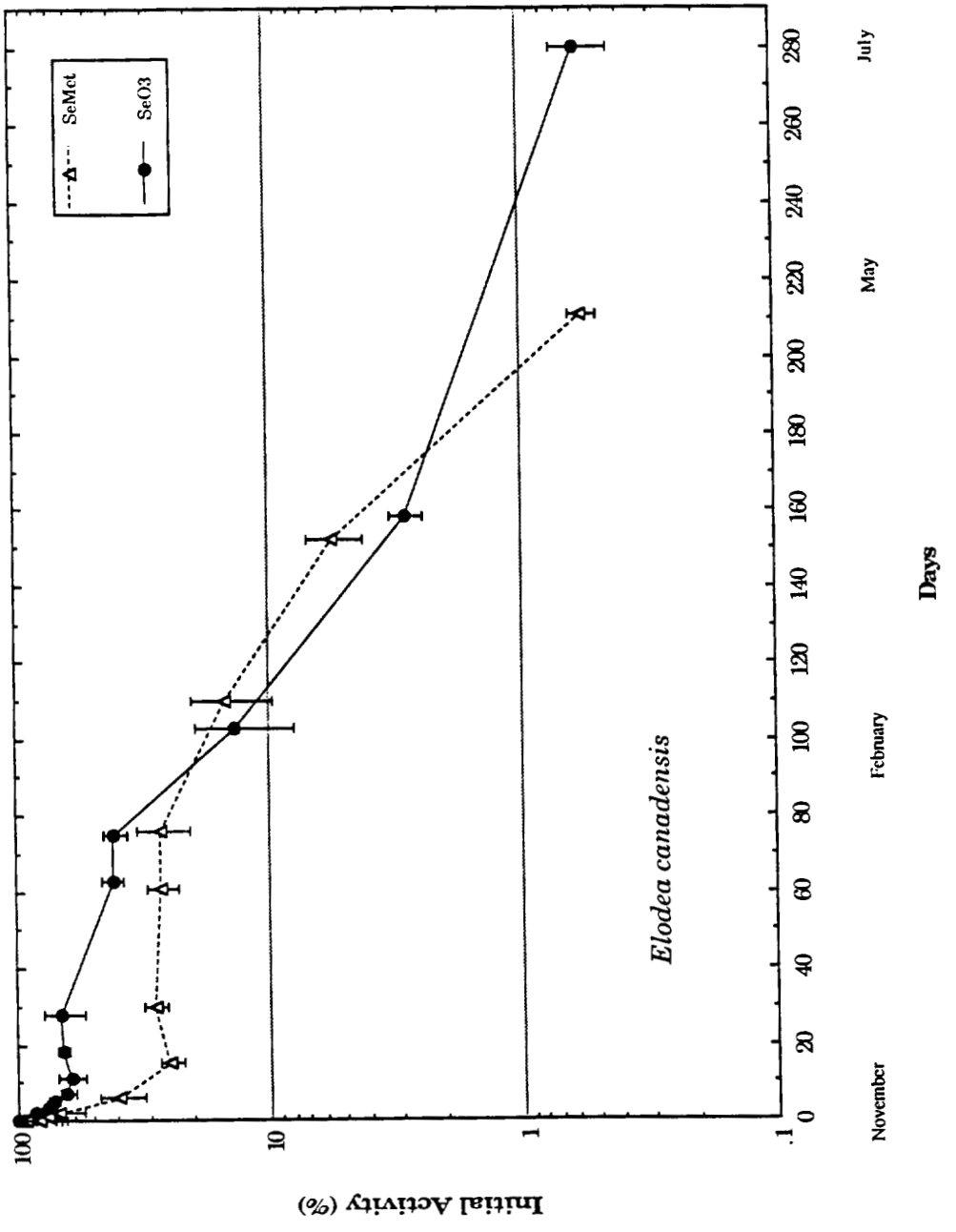

Fig. 3b.

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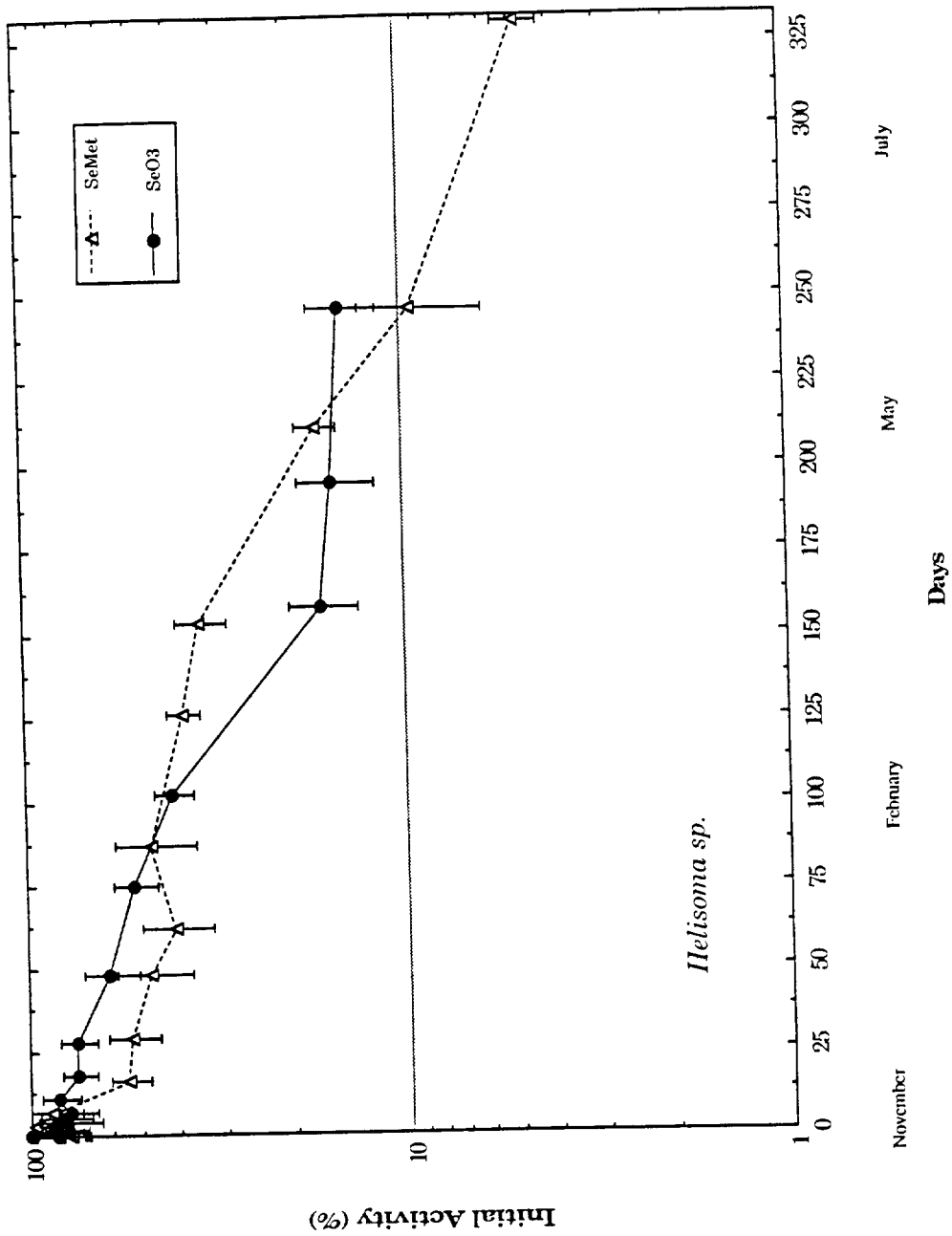

Fig. 3c

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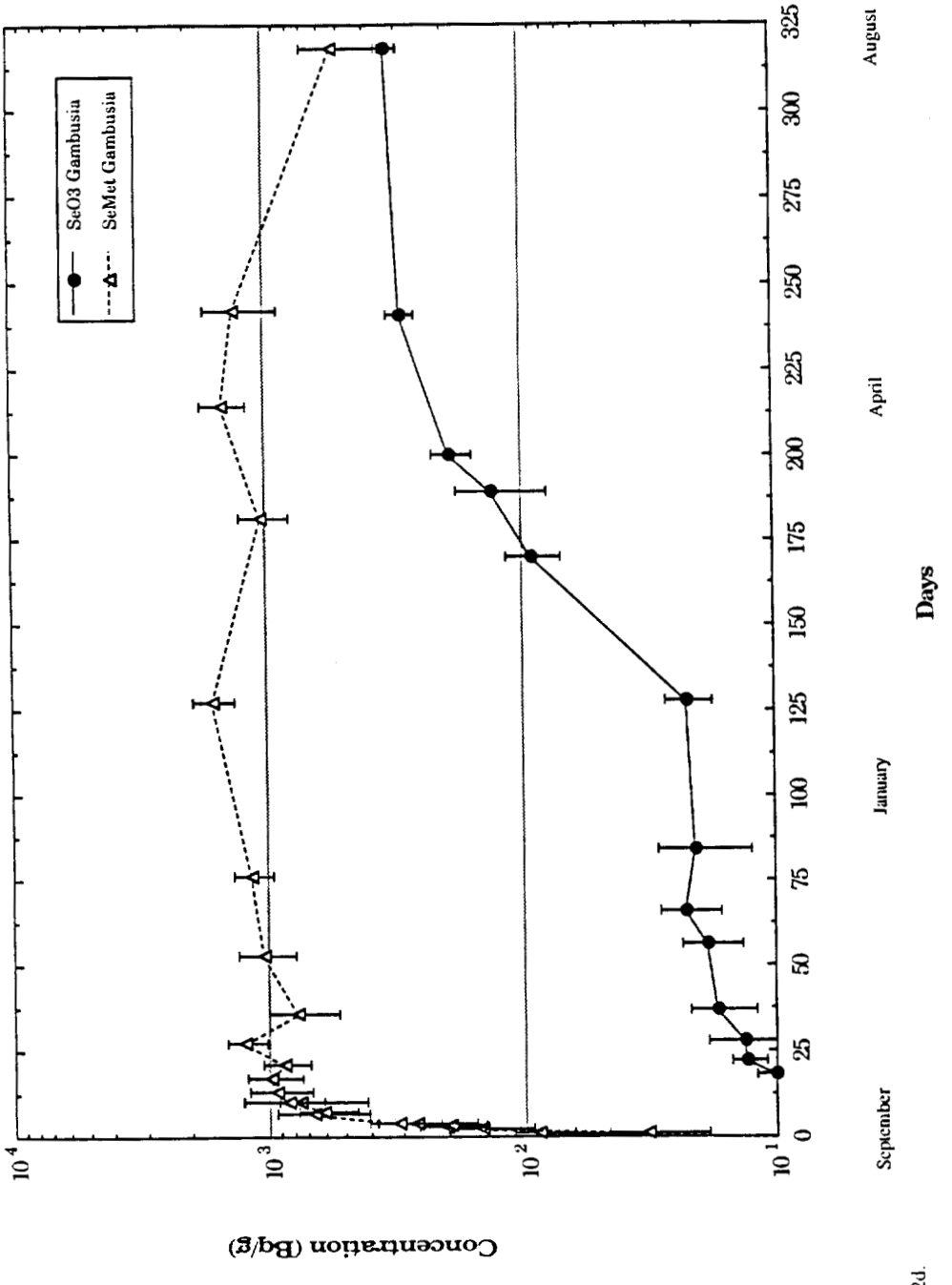

Fig. 2d.

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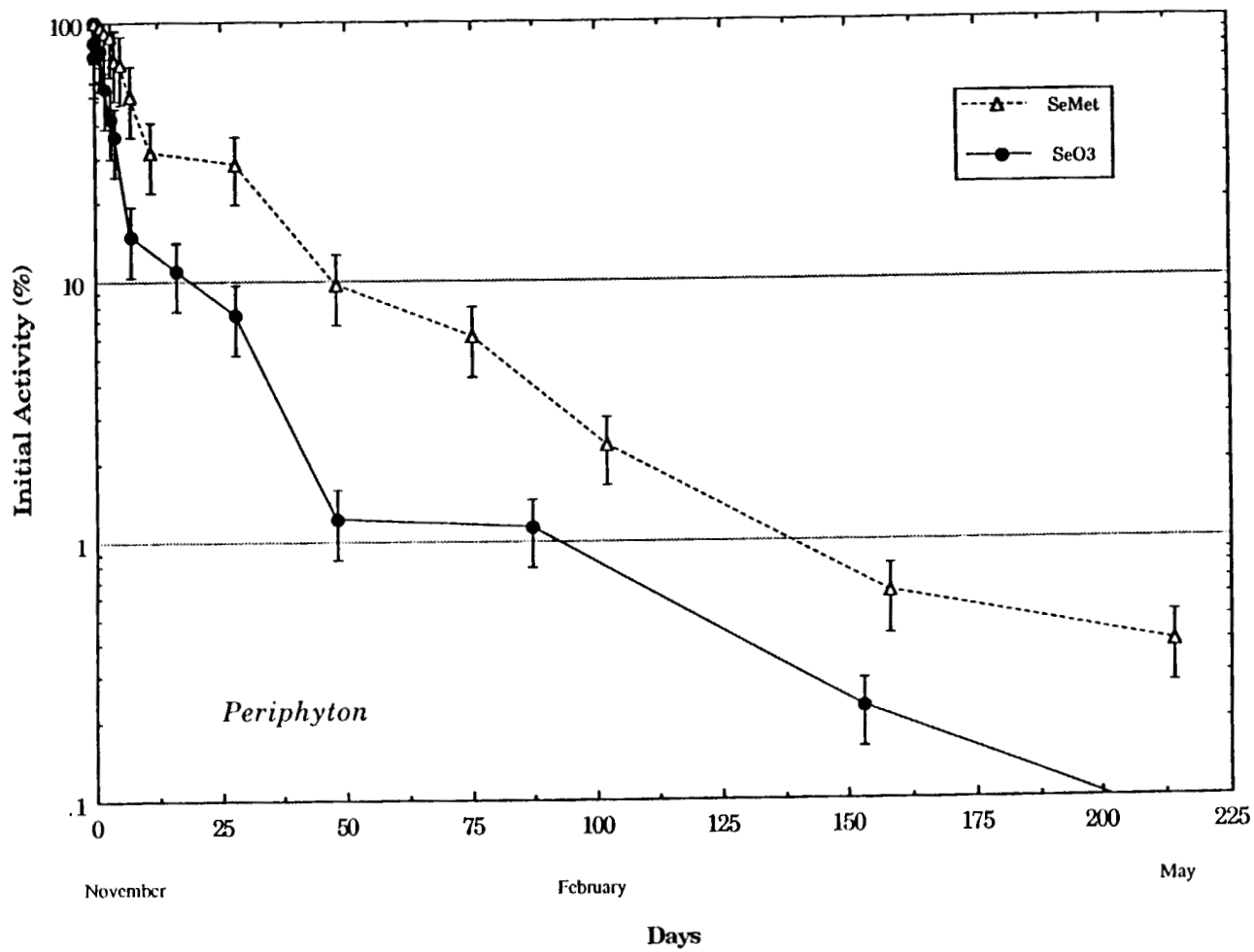

[illegible] Se eliminated per day by (a) *Periphyton*, (Bq g^{-1} dry weight); (b) *E. canadensis*, (Bq g^{-1} dry weight); (c) *Helisoma* sp. (Bq g^{-1} wet [illegible] (Bq g^{-1} wet weight) in experimental ponds spiked either with ^{75}Se-Selenite or ^{75}Se-SeMet (means ± 2 SE).

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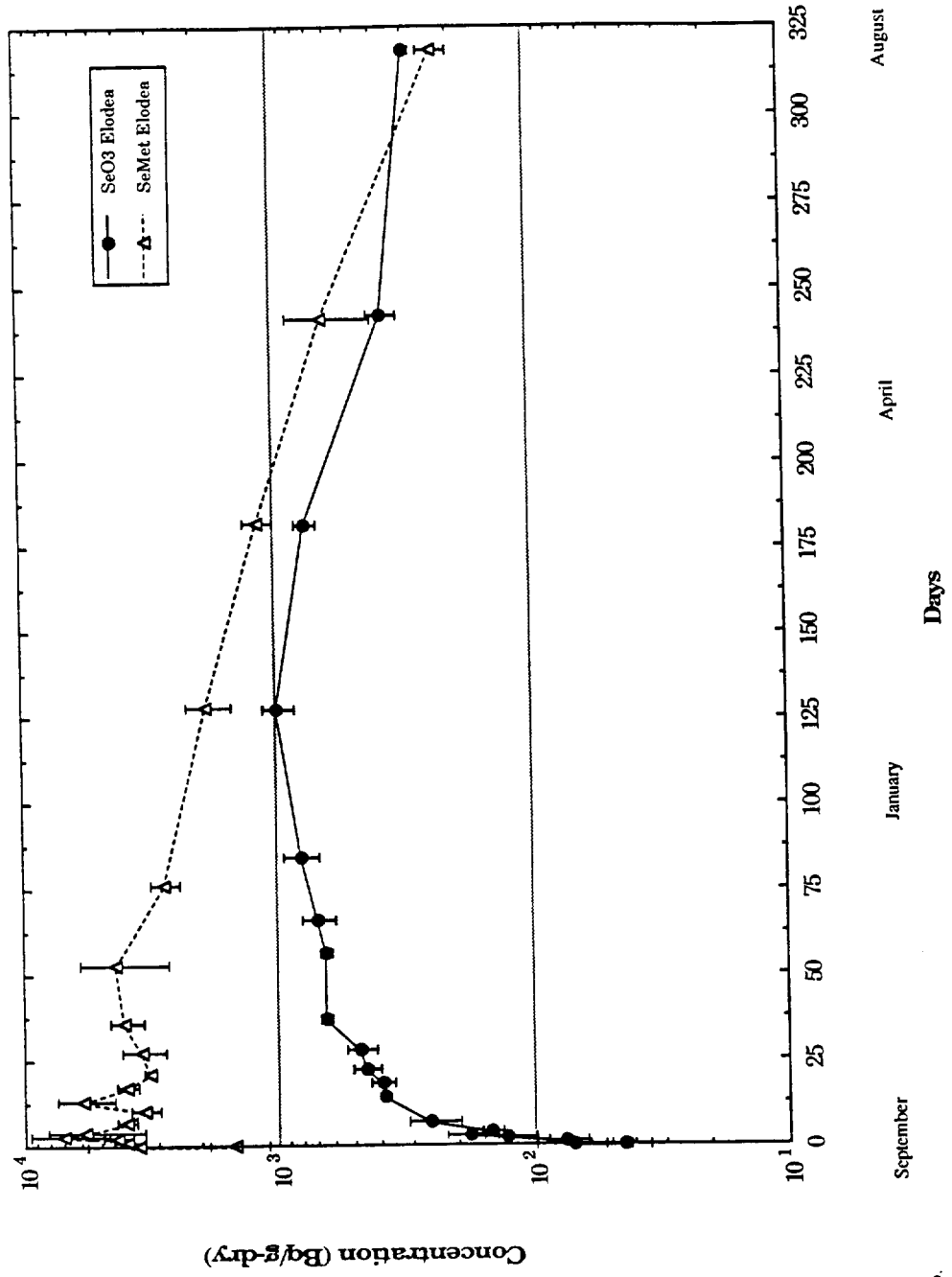

Fig. 2b.

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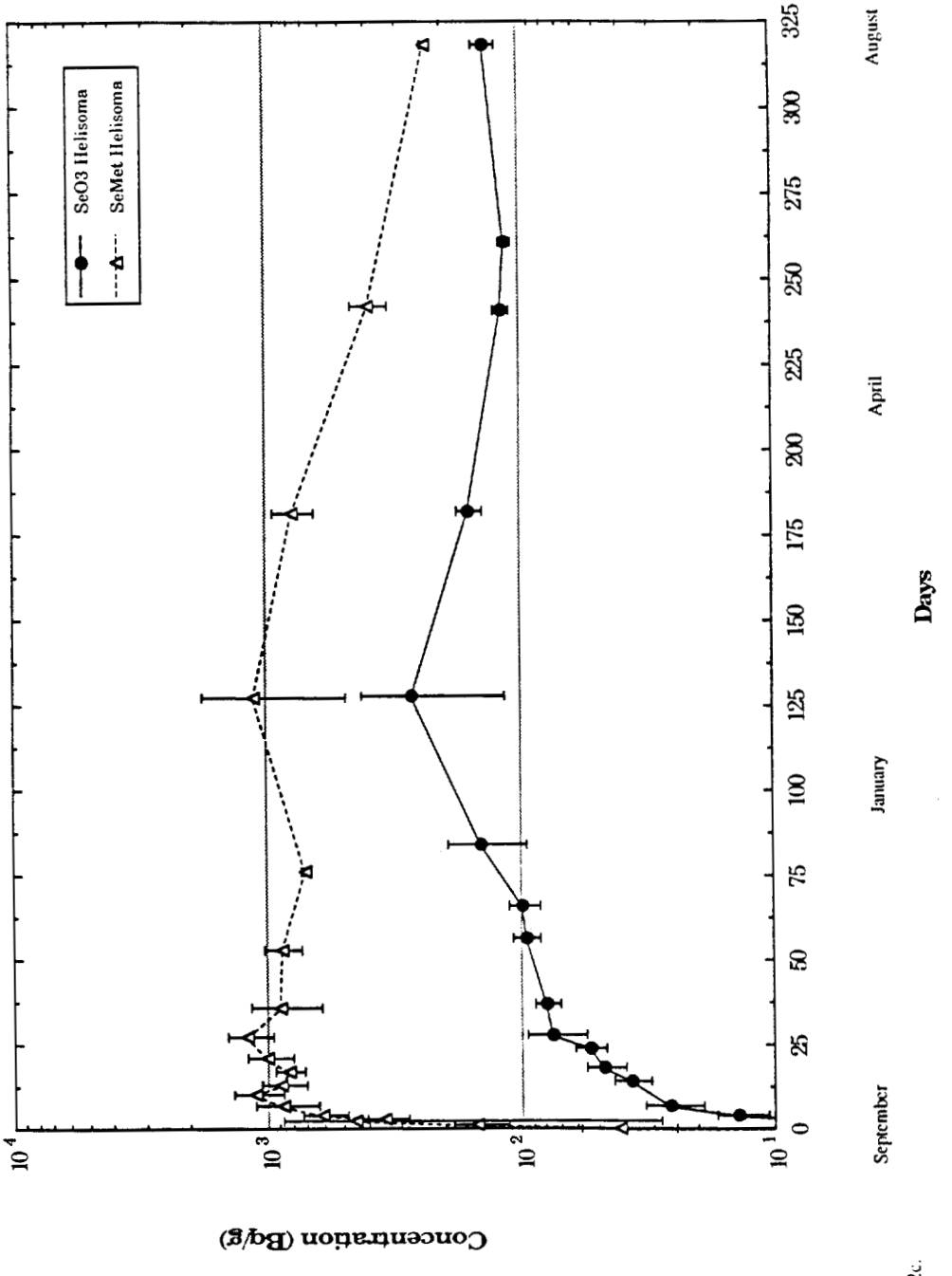

Fig. 2c.

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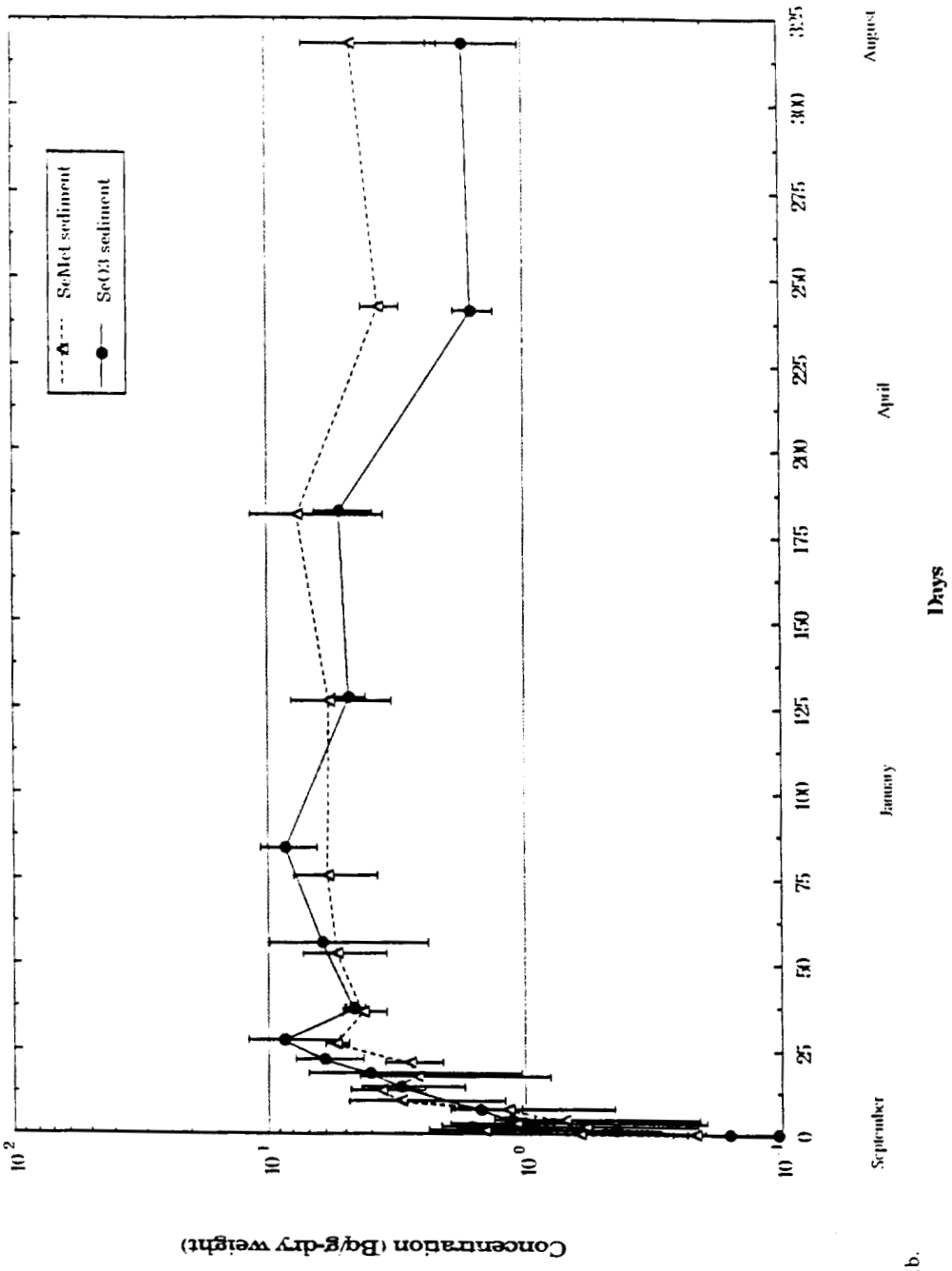

Fig. 1b.

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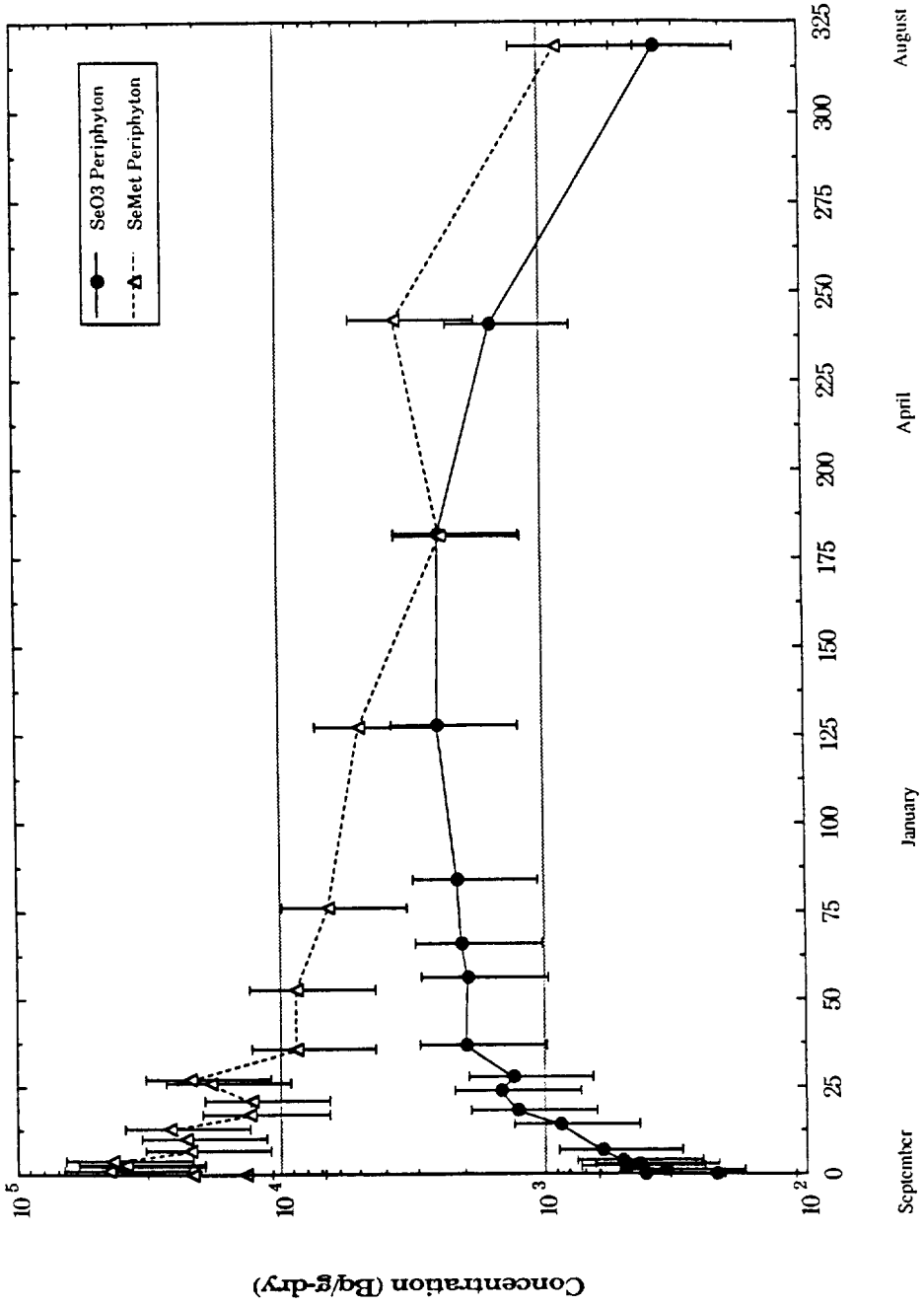

Fig. 2. ^{75}Se-Selenite and ^{75}Se-SeMet uptake by (a) *Periphyton*; (Bq g^{-1} dry weight); (b) *E. canadensis*, (Bq g^{-1} dry weight); (c) *Helisoma* sp. (Bq g^{-1} wet weight); and (d) G. affinis (Bq g^{-1} wet weight) through 318-d (means ± 2 SE).

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The samples were then filtered through a 0.45 μm Millipore filter and the filtrate analyzed for ^{75}Se. Fifty mL of the filtrate were gravity fed through both a 6.5 cm by 2.54 cm activated charcoal column and a 2.54 cm by 1.25 cm copper-chelex resin column (Cutter 1986). The filtrate from the charcoal column was then fed through a 5.1 cm by 2.54 cm cation exchange column (BioRad Dowex 50–100 Hydrogen mesh). The ^{75}Se activity of the filters and column filtrates were analyzed with a Packard NaI(Tl) Autolink Gamma Counter.

Forty-eight plexiglass slides (each 103 cm^2) were suspended in the water column of each pond to measure the uptake of the ^{75}Se by *Periphyton*. Two *Periphyton* slides were collected during each sampling period. Sediment cores (2.5 cm by 2.5 cm) and *E. canadensis* samples were collected from each of the quadrants per sampling period. *Periphyton*, *E. canadensis*, and sediment samples were analyzed for ^{75}Se content on a NaI(Tl) detector, wet weighed, dried (60 °C for 24 hr), re-weighed, and ashed (550 °C for 48 hr). Eight to 15 *Helisoma* sp. and *G. affinis* were collected from each pond per sampling period, live counted on the NaI(Tl) detector, wet weighed, and returned to their respective pond. All samples analyzed were decay corrected for the ^{75}Se 120-d radiological half-life. Concentration ratios (CR), the concentration of ^{75}Se in Bq g^{-1} of the biotic compartment/concentration of ^{75}Se in Bq g^{-1} of the water compartment, were determined at specific points in time during the experiment. A time-integrated concentration factor (CF) (Barry, 1979) was also determined at the end of the 318-d experiment ($\int^{75}Se$ in Bq g^{-1} of the biotic compartment/$\int$concentration of ^{75}Se in Bq g^{-1} of the water compartment). This additional step was necessary to account for Se uptake in the biota from a single acute spike rather than from a continuous chronic source.

Sixty days after ^{75}Se was added to the experimental ponds, 20 *Helisoma* sp., 25 *G. affinis*, 1.5 kg (wet wt) of *E. canadensis*, and 24 *Periphyton* slides from both experimental ponds were transferred to the reference pond for elimination studies. Samples in this elimination study were collected and analyzed as previously described. Biological half-lives of ^{75}Se-SeMet and ^{75}Se-Selenite were calculated for each biotic compartment from the slope of the long-term component of the log-transformed depuration curve using the formula: $T^{1/2} = 0.693/\lambda$, where λ is the slope of the linear regression curve from the plot of the natural logarithm of the ^{75}Se concentration versus time (Whicker and Schultz, 1982).

3. Results

Figures 1, 2 and 3 show the uptake and elimination of ^{75}Se for the various pond compartments throughout this study and are normalized to the same initial ^{75}Se activity (mean values ± 2 standard errors of the mean). Figure 1a depicts the ^{75}Se-Selenite and ^{75}Se-SeMet unfiltered and filtered water activity. The organic ^{75}Se left the water column more rapidly than the anionic ^{75}Se-Selenite species. However, sediment uptake of ^{75}Se was similar in the two experimental ponds (Figure 1b). The sediment/water distribution coefficient (K_d) was determined to be 2.2×10^4

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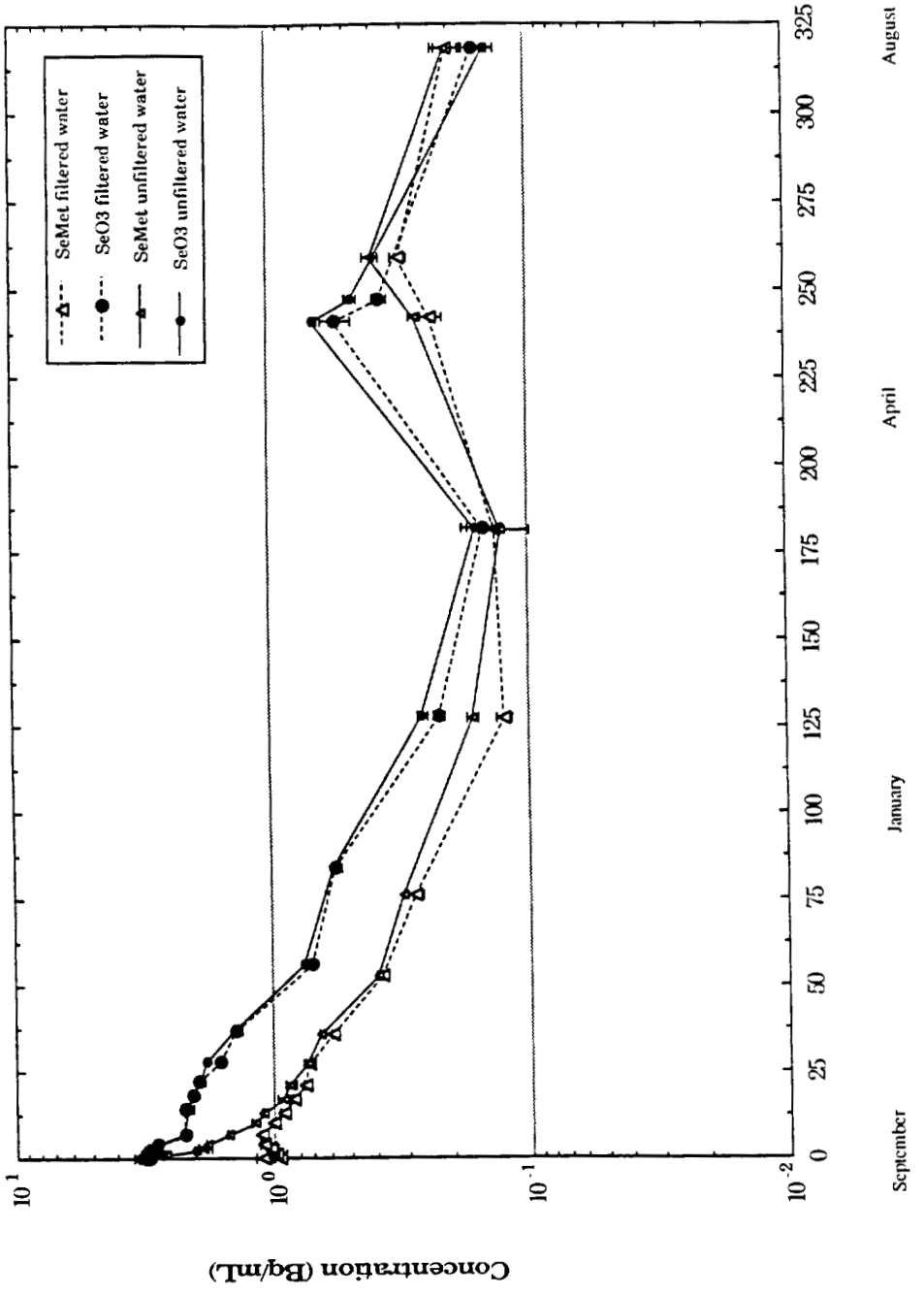

Fig. 1a. Unfiltered and filtered water ^{75}Se activity from ponds tagged with either ^{75}Se-Selenite or ^{75}Se-SeMet. (1b) Sediment ^{75}Se activity (Bq g^{-1} dry weight).

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