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UPTAKE OF CESIUM-134 BY THE EARTHWORM SPECIES *EISENIA FOETIDA* AND *LUMBRICUS RUBELLUS*

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Abstract—The uptake processes of ^{134}Cs in two earthworm species were investigated as well as the effect of temperature on these processes. The results show that equilibrium concentrations in the two species differ by 1.5- to fivefold. Equilibrium concentrations range from 367 to 963 Bq g^{-1} in *Lumbricus rubellus* and from 920 to 1,893 Bq g^{-1} in *Eisenia foetida*; biological half-lives range from 56 to 119 h and 52 to 64 h, respectively. Assimilation was two to four times higher in *E. foetida* and elimination rate one to two times higher in *E. foetida* than in *L. rubellus.* Further, the results show that temperature may affect the ^{134}Cs concentration in these earthworms by a factor of 1.4 to 2.1 between 10 and 20°C, depending on the species. The maximum difference found within one species was a factor of 2.6. Our results show no clear effect of temperature on the assimilation, but a small negative effect on elimination, resulting in an increasing biological half-life and concentration factor with higher temperatures.

Keywords—Earthworms ^{134}Cs Uptake process Temperature effects

INTRODUCTION

The radioisotopes ^{134}Cs and ^{137}Cs are among the most important radionuclides introduced into the environment as a result of nuclear tests in the early 1960s, and of nuclear accidents. Cesium has accumulated in the upper soil layers where it can be assimilated by plants and other organisms [1,2]. It can be concentrated in the food chain and therefore poses a considerable risk to humans, especially through the consumption of highly contaminated products such as mushrooms and reindeer meat.

Models calculating radionuclide transfer to humans are generally based on partition coefficients and concentration factors for crop and livestock to extrapolate soil concentrations to a dose. Such calculations do not always lead to reliable predictions of the dose as they are often based on 'best estimates' and neglect local circumstances. Partition coefficients, K_p's, as well as concentration factors are known to differ considerably between soil types [3]. Furthermore, concentration factors also depend on animal or crop species, and season and age of the deposition [4,5]. Identification of the most important environmental parameters determining the radionuclide transfer from soil to plants and animals would seem useful for predicting the behavior of a radionuclide more carefully.

Uptake of compounds by soil invertebrates is often assumed to be a function of the concentration in the liquid phase of the soil [6,7]. Environmental conditions may affect uptake by changing the equilibrium between solid and liquid phases, thus affecting the exposure concentration, and by directly affecting the uptake from the liquid phase. Temperature, for example, has a large effect on the metabolism of organisms and consequently on the uptake and excretion of radionuclides [8], but may also affect the transfer of radionuclides from liquid to solid phase and vice versa [9]. Potassium ions (K^+) and NH_4^+ ions affect the solid–solution equilibrium of Cs^+ [1], but are also known to have an effect on the uptake site at the cell wall [10,11]. In most accumulation studies little attention has been paid to the effects of environmental conditions on element uptake and the variation in concentration factors resulting from these alterations.

In the present study earthworms were used as test organisms. Earthworms have been used to study the uptake of uranium so as to predict the accumulation of uranium by other organisms and thus developing bioavailability indices for different soils [12]. It is generally assumed that earthworms absorb nutrients and elements directly from the liquid phase of the soil [6,13]. For this reason, several studies have been carried out on the accumulation of heavy metals and organic compounds from liquid medium by earthworms [14,15]. As do most other terrestrial invertebrates, earthworms show a relatively rapid turnover of cesium with a biological half-life of 2 to 4 d [16,17]. Consequently, results can be generated in a relatively short time.

The aim of this study was to determine the effects of different environmental parameters on the uptake and turnover of ^{134}Cs by soil organisms. The paper will describe the time-dependent uptake of ^{134}Cs from liquid medium by the earthworm species *Lumbricus rubellus* and *Eisenia foetida,* as well as the effect of temperature on the uptake process.

MATERIALS AND METHODS

Test Organisms

Experiments were carried out with two common earthworm species, *E. foetida* and *L. rubellus.* Individuals of *E. foetida,* which were at least six weeks old, were taken from a laboratory culture in which the earthworms were kept on horse manure at 20°C. Adult individuals of *L. rubellus* were purchased from a commercial dealer.

Experimental setup

The earthworms were kept on moist filter paper for 2 d to empty the gut before exposure to ^{134}Cs. The earthworms were then kept for 2 weeks in a liquid medium resembling ground

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Table 1.Change in ^{134}Cs concentration, pH, and conductivity of the media in the different treatments described by regression analysis

	Temp. (°C)	*E. foetida*			*L. rubellus*		
		p	$t = 0$	$t = 336$	*p*	$t = 0$	$t = 336$
Concentration (Bq ml^{-1})	10	0.000	51.0	52.6	0.736	50.5	
	15	0.000	49.5	54.0	0.000	49.2	53.4
	20	0.020	51.1	52.2	0.342	50.9	
pH	10	0.059	7.35		0.006	7.40	7.18
	15	0.000	7.79	8.22	0.027	7.76	7.57
	20	0.008	7.38	7.46	0.455	7.33	
Conductivity (mS/cm)	10	0.066	8.25		0.005	8.23	8.66
	15	0.000	7.46	8.71	0.000	7.43	8.55
	20	0.000	8.24	8.66	0.050	8.22	8.46

Values at the start ($t = 0$ h) and after 2 weeks ($t = 336$ h) are given in the case of a significant regression ($p \leq 0.05$), otherwise only the value at the start is given.

water to study the uptake of ^{134}Cs from the liquid phase of the soil. This setup has also been used to study the uptake of chlorobenzenes by *Eisenia andrei* [15]. The medium was prepared by adding 2.47 g NaCl, 1.89 g $MgSO_4 \cdot 7H_2O$, 0.039 g $CaCl_2 \cdot 2H_2O$, 0.111 g $NaNO_3$, 0.994 g Na_2SO_4, and 0.336 g $NaHCO_3$ to 1 L demineralized water. The pH of the medium was about 8.5. ^{134}Cs was obtained from Amersham (UK) (CCS1, CsCl in aqueous solution) and was added to the medium to a concentration of about 50 Bq ml^{-1}.

The experiments were carried out in climate chambers with a small light source and a humidity of 60 to 70%. In the first experiment the earthworms were kept individually at a temperature of 15°C in glass pots with 25 ml of medium. Six earthworms of both species were sampled randomly after 0, 1.5, 4, 6, 25, 50, 121, 218, and 336 h. In the second experiment earthworms were kept at 10 and 20°C in 50 ml medium. Five individuals of both species were sampled after 0, 1, 3, 5, 24, 48, 120, 216, and 336 h. After sampling, the earthworms were weighed and stored at −20°C. Prior to the measurements, they were dried at 80°C for 2 d and weighed. The samples were left for one night in a 1-ml mixture of HNO_3 (65%) : $HClO_4$ (70%) : H_2SO_4 (95%), 69:5:1. The next morning 3 ml of this mixture was added to these samples, which were heated for at least 40 min at 90°C. Subsequently, the temperature was increased 20°C every 30 min until reaching 210°C [18]. Cesium-134 concentration was measured on a Minaxi gamma counter after diluting the pellet in 10 ml demineralized water; measurements were corrected for decay to the starting date of the experiment. Conductivity, pH, and ^{134}Cs concentration of the medium were measured immediately after each sampling. To correct for small variations in exposure concentration the earthworm concentrations were normalized to an exposure concentration of 50 Bq ml^{-1}.

Modelling and statistics

The change in concentration, pH, and conductivity of the medium was tested by regression analysis. The dry weight of the earthworms sampled throughout the experiment at 15°C was also tested by regression analysis.

The equation for a linear one-compartment model was fitted to the data, using the Nonlin module of the SYSTAT statistical package. Fitting procedures for nonlinear regression generally use unweighed least squares, which assume homogeneity of variances and normal distribution of the residuals. However, accumulation curves are characterized by increasing concentrations with time and thus with increasing variation. To correct for bias due to higher variation at the end of the experiment, standard errors were used as a weighing factor in calculating the loss function. This procedure showed a larger influence of the data at the start of the experiment on the parameter values (M.P.M. Janssen, unpublished data).

A one-compartment linear model was fitted to the data; ^{134}Cs concentration in the earthworms at time *t* (Q_t) is given by the equation:

$$Q_t = \frac{A}{k}(1 - e^{-kt})$$

in which *A* is the assimilation (in Bq g^{-1} h^{-1}) and *k* is the elimination rate (h^{-1}). Equilibrium concentrations were calculated by substituting ∞ for *t*. Biological half-lives were calculated as $T_b = \ln 2/k$ [19].

RESULTS

Medium

The ^{134}Cs concentrations in the media at the start and at the end of the experiments are given in Table 1. The difference in pH and conductivity between the 15°C and the 10 and 20°C media may reflect small differences in constituents. Concentrations increased slightly in most treatments as a result of evaporation. The pH increased slightly in the experiments with *E. foetida,* whereas a small decrease was observed in those with *L. rubellus.* Conductivity increased significantly in all treatments but one, the highest increase being about 15%. The changes in concentration and conductivity were largest in the 15°C treatment, which can probably be attributed to the smaller volume of the medium in this experiment and hence the greater impact of evaporation.

Earthworms

There were no significant changes in dry weight of the earthworms with time. The mean dry weights of *E. foetida* and *L. rubellus* at 15°C were 0.037 and 0.132 g, respectively, and mean percentage dry weights were 12.5 and 14.1%, respectively. Comparable values were observed at 10 and 20°C. Cesium-134 concentrations were not significantly different from zero at the start of the experiments and clearly increased with time for both species at each temperature. Final concentrations differed considerably between *E. foetida* and *L. rubellus* as well as concentrations at 10 and 20°C (Figs. 1 and 2).

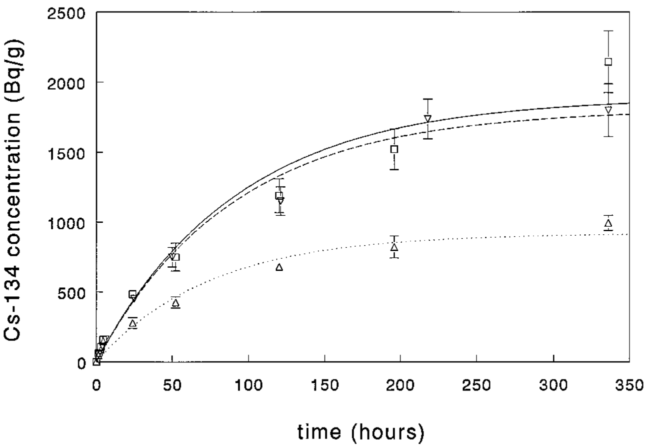

Fig. 1. Uptake of ^{134}Cs from liquid medium (50 Bq ml^{-1}) by the earthworm *Eisenia foetida* at 10, 15, and 20°C (in Bq g^{-1} dry weight). The data are given as means plus standard errors, △ = 10°C, ▽ = 15°C, □ = 20°C. The data are fitted using a one-compartment model; dotted line = fit at 10°C, dashed line = fit at 15°C, and solid line = fit at 20°C.

The results of the modelling (Figs. 1 and 2) indicated that uptake (*A*) is much higher in *E. foetida* than in *L. rubellus,* whereas elimination is similar or lower. *Eisenia foetida* accumulated between 12 and 20 Bq g^{-1} h^{-1} and *L. rubellus* about 5 Bq g^{-1} h^{-1}. Elimination is about 1.2% per hour in the first species and between 0.6 and 1.2% per hour in the second (Table 2). Equilibrium concentrations calculated from the accumulation (*A*) and the elimination rate (*k*) are 367 to 963 Bq g^{-1} in *L. rubellus* and 920 to 1,893 Bq g^{-1} in *E. foetida*; biological half-lives are 56 to 119 h and between 52 to 64 h, respectively (Table 2). Concentration factors calculated from equilibrium concentrations are higher in *E. foetida* than in *L. rubellus* and range from 18 to 38 and from 7 to 19, respectively.

Higher equilibrium concentrations were observed in both species at 20°C than at 10 and 15°C (Table 2 and Figs. 1 and 2). The parameter estimation shows an increase in the assimilation and a small decrease in the elimination with temperature in *E. foetida.* As a result, a considerable increase in concentration with temperature is observed (Table 2). The pattern in *L.*

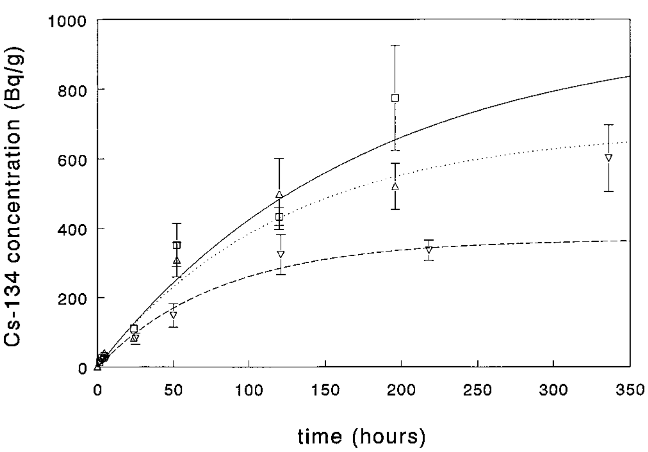

Fig. 2. Uptake of ^{134}Cs from liquid medium (50 Bq ml^{-1}) by the earthworm *Lumbricus rubellus* at 10, 15, and 20°C (in Bq g^{-1} dry weight). The data are given as means plus standard errors, △ = 10°C, ▽ = 15°C, □ = 20°C. The data are fitted using a one-compartment model; dotted line = fit at 10°C, dashed line = fit at 15°C, and solid line = fit at 20°C.

Table 2. Parameter estimates for ^{134}Cs uptake from liquid medium by the earthworms *E. foetida* and *L. rubellus* at different temperatures using a one-compartment model (*A* and *k*)

	Temp. (°C)	*A* (bq g^{-1} h^{-1})	*k* (h^{-1})	Equil. (Bq g^{-1})	T_b (h)	CF (Bq g^{-1}/ Bq ml^{-1})
E. foetida	10	12.4	0.013	920	52	18.4
	15	20.1	0.011	1797	62	35.9
	20	20.5	0.011	1893	64	37.9
L. rubellus	10	5.62	0.008	688	85	13.8
	15	4.55	0.012	367	56	7.3
	20	5.59	0.006	963	119	19.3

Equilibrium concentration (Equil.), biological half-life (T_b), and concentration factor (CF) are derived from the parameters *A* and *k.* CFs represent the ratio of the estimated equilibrium concentration (Bq/g dry weight) and the concentration in the medium (Bq/ml). The results at 10 and 20°C were obtained in one experiment, the results at 15°C in another experiment.

rubellus is less clear as there is no effect of temperature on uptake and the elimination is highest at 15°C. Consequently, a lower equilibrium concentration is observed at 15°C (Table 2). The results show that a temperature shift of 10°C may affect the ^{134}Cs concentration in these earthworms by a factor of 1.4 to 2.1, depending on the species. Highest and lowest ^{134}Cs concentration within one species differed by a factor of 2.6, in *L. rubellus* kept at 15 and 20°C, respectively. Maximum observed difference between the two species was 4.9, observed at 15°C.

DISCUSSION

Data on biological half-lives of cesium have been summarized by DiGregorio et al. [17] and by Yates and Crossley [20]. Additional data have been summarized in Table 3. Cesium is easily assimilated by soil invertebrates; assimilation rates generally exceed 60 to 70% of the ingested amount [20,21]. Cesium is characterized by a moderate to rapid elimination rate compared to some other elements, such as iodine; consequently biological half-lives are relatively short. Elimination rates of cesium vary among soil invertebrates, which is thought to be related to their physiology [20,21]. In general, biological half-lives of cesium are relatively long for centipedes and isopods, whereas in earthworms and most insects a rapid turnover is observed; millipedes show intermediate values. Our data are comparable to the biological half-lives of cesium for three earthworm species calculated by Crossley et al. [16], which range from 48 to 117 h (Table 3). The relatively high elimination rate suggests absence of net accumulation with age, as might be the case in centipedes in which ^{134}Cs is eliminated slowly [20]. The differences in biological half-lives are reflected in the cesium concentrations observed under field conditions in different species [22–24].

Concentration factors are generally calculated by dividing the concentration in the organisms by the concentration in their environment. In this study the concentration factors for *E. foetida* and *L. rubellus* are calculated using the concentration in liquid medium. As the availability of elements in soil is generally smaller than in water, it is expected that these concentration factors will be higher than those calculated directly from soil. For comparison with concentration factors calculated from studies carried out in soils, the results given in Table 2 have been multiplied by default K_p values as given by Sheppard and Thibault [3]. This estimation results in concentration factors of 0.14 and 0.02 for *E. foetida* and of 0.07 and 0.01 for *L. rubellus*

Table 3.Parameter values for cesium kinetics for terrestrial invertebrates separate from those given by DiGregorio et al. [17]

Species	Temp. (°C)	T_b (h)	CF	References
Earthworms				
Octolasium lacteum	16	117		[16]
Lumbricus terrestris	16	48		
Eisenia hortensis	16	67		
Eisenia foetida	15	62	35.9	This study
	10	52	18.4	
	20	64	37.9	
Lumbricus rubellus	15	56	7.3	This study
	10	85	13.8	
	20	119	19.3	
Centipedes				
Scolopocryptops nigridia	20	874		[20]
Millipedes				
Dixidesmus erasus	20	58		[22]
Isopods				
Armadillidium vulgare	20	554	0.76	[8]
Cylisticus convexus	20	449	0.62	
Armadillidium nasatum	20	694	0.88	
Hepotonothrus pruinosus	20	332	0.71	
Insects				
Aedes aegypti	20	43		[35]
Acheta domesticus	29.4	62		[21]
Acheta domesticus	20	99		[31]
	25	95		
	30	74		
Romalea microptera	21.1	96		[30]
Lochmaea suturalis	Field	28.8	0.30	[36]
Ulopa reticulate	Field	456	0.48	

The concentration factors for *E. foetida* and *L. rubellus* are based on concentrations in liquid medium; the others are based on soil.

for clay and sand, respectively. Comparable values have been reported by Helmke et al. [25] for stable cesium, ranging from 0.02 to 0.04 and by Kålås et al. [26], ranging from 0.01 to 0.04. However, somewhat higher values, 0.28 to 0.92, have been mentioned by Rudge et al. [24]. Reichle et al. [27] reported values ranging between 3.6 and 56.9, but these are only based on soil organic matter and thus are not comparable with our data given above. The higher concentration factors found for different isopod species (see Table 3) clearly reflect the much higher biological half-life for ^{137}Cs in isopods.

The two earthworm species differ by a factor of one-and-a-half to five in equilibrium concentrations when applying the one-compartment model; concentrations are higher in *E. foetida* than in *L. rubellus* at each temperature (Table 2). Comparable differences were obtained with *E. foetida, E. andrei,* and *L. rubellus* in an unreported preliminary experiment and in experiments using different cations in the liquid medium [28]. Biological half-lives were generally longer in *L. rubellus* than in *E. foetida*. The higher accumulation of ^{134}Cs by *E. foetida* may be related to the much higher ratio volume to surface area ratio and the higher metabolic activity of this species. The species differ also in the metabolism of cesium analogues such as potassium and ammonium. However, a budget study, which can reveal a causal relationship between the concentrations of these compounds, has not yet been carried out ([1,28] M. Janssen, unpublished data).

Temperature is considered to be one of the most important abiotic factors affecting element turnover through organisms [8]. It may affect the assimilation as well as the elimination of ^{134}Cs, the net result depending on the effect on both. Crossley and Pryor [29] suggested that a higher temperature would result in a higher daily uptake, but also in a more rapid elimination. Experimental results, however, showed different patterns, taking into consideration the effect of temperature on assimilation, elimination, and equilibrium concentrations. Our results showed increasing assimilation (A), but a small negative effect on elimination (k) between 10 and 20°C for *E. foetida* (see Table 2). In contrast, assimilation decreased with temperature in crickets, whereas elimination increased [30]. Reichle [8] too found increasing elimination rates with higher temperatures in isopods. He attributed the higher cesium elimination to a more rapid excretion. In contrast to *E. foetida*, lowest assimiliation and highest excretion in *L. rubellus* was observed at 15°C. The difference in temperature optimum, 15 to 18°C for *L. rubellus* and 20 to 25°C for *E. foetida,* may be responsible for the differences observed between the two earthworm species [13,29,31]. The equilibrium concentration for *E. foetida* and *L. rubellus* increased 1.4 to 2.1 times when the surrounding temperature changed from 10 to 20°C, which corresponds with the general Q_{10} range. No positive correlations were found between temperature and ^{137}Cs concentrations in different algae species, whereas freshwater shrimp showed decreasing net uptake with a temperature between 20 and 32°C [32,33]. The results suggest that to predict the effect of temperature the processes of assimilation and elimination should be considered separately as well as the optimum temperature of the species.

Insight into the variation of the accumulation potential of species may contribute to a better prediction of the risks of contaminants and to identification of the species groups that are at risk. The results show that a 1.3- to 4.9-fold difference in ^{134}Cs concentration can be expected within the earthworm taxon at one temperature. Elimination and biological half- lives differ by a factor of two to three among the earthworm species (Tables 2 and 3). Maximum difference in ^{134}Cs concentration between two experimental groups was around 10-fold. Reichle [8] mentions a manifold difference in elimination coefficient in closely related isopod species, but concludes that the coefficient is more similar in species from the same genus. Variation in sensitivity for toxic compounds was estimated to be less than a factor of 10 in different earthworm species, and depends on the compound studied [13]. It is apparent that even among closely related earthworm species, large differences in ^{134}Cs concentration may appear under similar experimental conditions. The results suggest that uptake of radionuclides may differ at least by several dozen-fold among soil organisms kept under comparable conditions.

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