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BIOTIC INTERACTIONS MODIFY THE TRANSFER OF CESIUM-137 IN A SOIL–EARTHWORM–PLANT–SNAIL FOOD WEB

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Abstract—The present study investigated the possible influence of the earthworm *Aporrectodea tuberculata* on the transfer of cesium-137 (^{137}Cs) from a contaminated (130 Bq/kg) deciduous forest soil to the lettuce *Lactuca sativa* and to the snail *Cantareus aspersus* (formerly *Helix aspersa*) in two laboratory experiments. In the first experiment, the International Organization for Standardization 15952 test was used to expose snails for five weeks to contaminated soil with or without earthworms. In these conditions, the presence of earthworms caused a two- to threefold increase in ^{137}Cs concentrations in snails. Transfer was low in earthworms as well as in snails, with transfer factors (TFs) lower than 3.7×10^{-2}. Activity concentrations were higher in earthworms (2.8–4.8 Bq/kg dry mass) than in snails (<1.5 Bq/kg). In the second experiment, microcosms were used to determine the contribution of soil and lettuce in the accumulation of ^{137}Cs in snails. Results suggest that the contribution of lettuce and soil is 80 and 20%, respectively. Microcosms also were used to study the influence of earthworms on ^{137}Cs accumulation in snail tissues in the most ecologically relevant treatment (soil–earthworm–plant–snail food web). In this case, soil-to-plant transfer was high, with a TF of 0.8, and was not significantly modified by earthworms. Conversely, soil-to-snail transfer was lower (TF, ~0.1) but was significantly increased in presence of earthworms. Dose rates were determined in the microcosm study with the EDEN (elementary dose evaluation for natural environment) model. Dose rates were lower than 5.5×10^{-4} mGy/d, far from values considered to have effects on terrestrial organisms (1 mGy/d).

Keywords—Radioecology Radiocesium Dosimetry Food chain Bioavailability

INTRODUCTION

Cesium-137 (^{137}Cs) is one of the most frequent artificial radionuclides found in European soils because of different anthropogenic discharges, such as atmospheric nuclear weapons testing, accidental releases from nuclear power plants, and chronic emissions from nuclear reactors and fuel-reprocessing plants [1,2]. When releases have occurred in the atmosphere, condensed aerosols of the more volatile elements (cesium, iodine, and ruthenium) have been released worldwide [3]. The distribution of radiocesium in the environment is then related to the chemical properties of Cs^+, which generally dictate a high degree of mobility and bioavailability of the radionuclide [1]. Cesium-137 is an emitter of gamma and beta radiation, with a long radioactive half-life (30.17 years) compared to human life expectancy. Because of these characteristics, ^{137}Cs may be toxic to human health and wildlife, not because of direct chemical toxicity of Cs ions but, rather, because of its radiation, which may lead to physiological and genetic damages. Depending on the radiosensitivity of the considered organism and of the dose that it receives, ionizing radiation causes mortality, decrease of growth and of reproductive capacities, or chromosomal damage [4]. Deleterious effects of ^{137}Cs exposure on various organisms (plants, invertebrates, and vertebrates) have already been shown [5]. Hence, numerous studies have investigated radiocesium fate in the environment and the subsequent risks for living organisms [6].

The improvement of current risk assessment procedures notably requires a better knowledge of the mechanisms involved in the transfer of ^{137}Cs in food webs, including the parameters that may affect its bioavailability [7]. Most radioecological works have focused on the influence of abiotic factors (mainly soil physicochemical characteristics) on ^{137}Cs bioavailability [8]. The role of biotic factors like earthworm activity, however, should not be neglected. Earthworms are, indeed, considered to be engineer soil organisms that have a major importance in soil functioning [9]. They play a role in the incorporation, fragmentation, and decomposition of organic matter (OM) as well as in humus formation, in soil porosity (and then water fluxes), and in the distribution and bioavailability of major nutrient elements (nitrogen, potassium, etc.) [10]. Bioturbation also has been shown to influence the vertical and horizontal distribution of various contaminants, including ^{137}Cs [11]. As a consequence, earthworms might have an effect on the contamination of other organisms. For instance, Wen et al. [12] showed that the activity of *Eisenia fetida* worms increased the mobility and the bioavailability of various heavy metals to wheat (*Triticum aestivum*). In a study on the Chinese cabbage *Brassica rapa*, inverse results were found, with *E. fetida* activity decreasing the bioaccumulation of cadmium and copper in the plant [13]. The only study that we found dealing with the transfer from soil to animal showed

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that *Aporrectodea tuberculata* significantly increased the transfer of cadmium, copper, and zinc from a soil to snails (*Cantareus aspersus*) [14]. To our knowledge, the influence of earthworms on ^{137}Cs bioavailability has never been studied.

Soil animals can be contaminated by three routes of exposure (digestive, dermal, and/or respiratory) and various sources (inorganic or organic soil particles, living or dead plant material, etc.), which make understanding of the mechanisms of soil-to-animal transfer difficult. Avery [1] suggests that the most important route of ^{137}Cs accumulation in animals is consumption of contaminated food. Diet is, indeed, thought to have the main role in intra- and interindividual differences in contamination levels [2]. Accurate data about the contribution of different sources in the total ^{137}Cs contamination in an organism, however, are scarce.

In recent years, studies and initiatives from international organizations dealing with protection of nonhuman biota against ionizing radiation have increased, showing a need for tools and knowledge in radioecological risk assessment [15]. Among the identified requirements, the need for additional information regarding the biological effects of radiation at environmentally significant dose rates has been emphasized [16]. Calculation of dose rates is an essential step in risk assessment procedures, but even though concepts are well defined, application still needs to be improved [16,17]. To date, dosimetric models available for nonhuman biota are much less sophisticated than those available for humans [15,17]. Notably, current models do not allow dose calculation whatever the organism and the situation of exposure [18]. The newly developed EDEN (elementary dose evaluation for natural environment) model attempts to resolve this lack of flexibility and aims at calculating dose rates for every nonhuman organism under many different exposure pathways [18].

The first aim of the present study was to assess the influence of *A. tuberculata* activity on the transfer of ^{137}Cs from soil to plant (the lettuce *Lactuca sativa*) and from soil to invertebrate (the brown garden snail, *C. aspersus*). *Aporrectodea tuberculata* is an endogeic earthworm that impacts decomposition activity, nutrient mineralization, and primary production and that frequently is used for ecotoxicological experiments. The terrestrial gastropod *C. aspersus* is an herbivorous and detritivorous organism, eating OM, fungi, living plants, and soil. It thus integrates these different sources of exposure. *Cantareus aspersus* is one of the most commonly used snail species in ecotoxicology because of its capacity to accumulate inorganic contaminants. Some studies successfully investigated its potential use as a biomonitor of radioactive contamination [19,20]. Moreover, this species is a prey item for many predators, and it is even consumed by humans in some countries. Thus, snails may constitute a vector of ^{137}Cs through food webs. The lettuce was chosen as a plant model, both because it could be of concern in human intoxication by ^{137}Cs after an accidental release of radionuclides [21] and because this plant is well accepted as a food source by snails. The second aim of the present study was to estimate the relative contribution of biotic (plant) and abiotic (soil) sources in the total accumulation of ^{137}Cs in snail tissues. Finally, we aimed to calculate the dose rates with the EDEN model [18].

MATERIALS AND METHODS

Organisms and soils

Snails (*C. aspersus*, formerly *Helix aspersa*) came from our standardized laboratory rearing. The individuals were aged for one month and weighed 0.85 ± 0.13 g (fresh mass; mean ± standard deviation throughout). The seeds of the lettuce *L. sativa* var. Reine de Mai came from organic farms and were marketed by Vilmorin-Oxadis (Saint Quentin Fallavier, France). Adult earthworms (*A. tuberculata*) produced for fishing came from Mondial Pêche (Besançon, Franche-Comté, France). One hundred kilograms of a leptosol (Food and Agriculture Organization classification) soil was sampled in an oak and hornbeam forest of Franche-Comté (northeastern France; altitude, 710 m; calcareous substratum from glacial deposit). The main physicochemical characteristics of this soil were as follows: pH-H_2O 5.48 and pH-KCl 4.86 [22]; cation-exchange capacity, 28 cmol/kg [23]; 45% clay [24], 10% sand [24], and 12% OM [25,26]; organic carbon, 66.9 g/kg [25,26]; total nitrogen, 4.38 g/kg [25,26]; C:N, 15.3 [25,26]; NH_4-N, 65.4 mg/kg [27]; potassium, 8.3 g/kg [28]; field capacity, 58%. The ^{137}Cs activity concentration was 130 ± 4 Bq/kg dry mass. Activity concentration of ^{137}Cs was measured using gamma spectrometry (see below). The soil was manually sorted to take off roots, stones, and macrofauna and then sieved (mesh size, 5 mm). Because a litter was present on the soil sampling site, it also was sampled and used in experiments. The litter layer was a mull (a forest humus without a H layer and with an A1 layer gradually merging into the mineral soil beneath) made up of *Quercus robur petraea* (50%), *Carpinus betulus* (30%), *Fraxinus excelsior* (10%), *Acer campestre* (5%), *Sorbus aria* (4%), and *Fagus sylvatica* (1%). Soil and litter samples were frozen (−20°C, 48 h) and then heated (60°C, 48 h) in a drying oven to eliminate most of the fauna. Litter samples were homogenized before their use in experiments. After the experiment, soil pH-H_2O was measured according to the International Organisation for Standardization standard 10390: 1994 [22], and OM content was assessed by ashing at 550°C for 5 h [29].

Experimental design

Two different experimental designs were used. The first one was a standardized design [30], allowing determination of the effects of soil contamination on juvenile (age, one month) land snail growth. Briefly, snails (n = 5 individuals) were reared in transparent polystyrene containers of 3,200 cm^3 (E1DBBAC001; Charles River Laboratory, L'Arbresle, France). Snails were exposed according to four experimental treatments (Table 1) with four replicates each. Treatments involved exposure to soil with or without litter and/or earthworms. The treatments will be called hereafter CS (container + soil), CSL (container + soil + litter), CSE (container + soil + earthworms), and CSLE (container + soil + litter + earthworms). Comparisons of all those treatments will allow estimation of the effect of earthworms on ^{137}Cs transfer and the relative contribution of litter and soil to ^{137}Cs bioaccumulation in snail tissues (as described further). Each container with earthworms was filled with 440 g (dry mass) of soil, and three earthworms weighing 5.23 ± 1.59 g (fresh mass) were introduced. Containers without earthworms contained 140 g of soil. Soil was watered to maintain soil moisture at 40% of its field capacity. In CSL and CSLE treatments, 5 g (dry mass) of the litter sampled in the field was homogeneously deposited on the soil surface. The containers were randomly placed in a controlled-conditions chamber at the beginning of the experiment, and their place was randomly changed three times a week.

In the containers, snails were fed ad libitum three times a

Table 1. Experimental designs and treatments

Experimental design	Treatment[a]	No. of replicates	No. of snails	No. of earthworms	Litter	Exposure duration (weeks)	
						Snails	Earthworms
Container	CS	4	5	0	No	5	0
	CSL	4	5	0	Yes	5	0
	CSE	4	5	3	No	5	5
	CSLE	4	5	3	Yes	5	5
Microcosm	MS	4	15	0	No	8	12
	MSP	5	15	0	No	8	12
	MSPE	7	15	8	No	8	12

[a] CS = experiments made in containers with contaminated soil; CSL = experiments made in containers with soil and contaminated litter; CSE = experiments made in containers with soil and earthworms; CSLE = experiments made in containers with soil, contaminated litter, and earthworms; MS = experiments made in microcosms with soil; MSP = experiments made in microcosms with soil and plant; MSPE = experiments made in microcosms with soil, plant, and earthworms.

week with leaves of lettuce cultivated in an unpolluted substrate made of 50% compost and 50% uncontaminated brown soil (Food and Agriculture Organization classification: Luvisol). Lettuce was cultivated in a controlled-conditions chamber with a 14:10-h light:dark photoperiod (light intensity, 150 μmol photons/m^2/s) at 24°C during the day and 16°C during the night. The snails were exposed for five weeks in a controlled-conditions chamber with a 18:6-h light:dark photoperiod (light intensity, 150 μmol photons/m^2/s). The temperature was 24°C during the day and 16°C during the night. The containers were cleaned (snail feces and mucus were removed from the top and the sides of the containers) three times a week throughout the experiment. When necessary, soils were rewatered to maintain their moisture at its initial level.

The second exposure design was a more ecologically representative microcosm, similar to that recently used by Scheifler et al. [31]. Snails were exposed in stainless-steel, cylindrical microcosms (diameter and height, 0.25 m) filled with approximately 5 cm of carefully washed gravel (to constitute a draining layer) and 5 kg (dry mass) of soil. Each microcosm was placed on a plant tub. A lid made of a polyvinyl chloride structure and a glass-fiber netting (mesh size, 1 mm) was fitted securely over the top of the microcosms. Three experimental treatments were carried out (Table 1) with four to seven replicates each. The treatments will be called hereafter MS (microcosm + soil), MSP (microcosm + soil + plant), and MSPE (microcosm + soil + plant + earthworms. The MS and MSP treatments allowed evaluation of the relative contribution of soil and lettuce in the total accumulation of ^{137}Cs in snail tissues, and the comparison of the MSP and MSPE treatments allowed assessment of the possible influence of earthworms on ^{137}Cs transfer from soil to snail and to lettuce. In the MSP and MSPE treatments, 15 lettuce seeds were planted in each microcosm, and after eight weeks of cultivation, only the three sturdiest plants were kept; the other seedlings were removed from the microcosms. In the MSPE microcosms, eight adult earthworms weighing 4.50 ± 1.16 g (fresh mass) were introduced.

After 12 weeks of cultivation, 15 juvenile snails were introduced in each microcosm and exposed for the following eight weeks. The total exposure duration for earthworms was thus 20 weeks. In MSP and MSPE microcosms, the snails could consume both soil and lettuce grown on it. In MS microcosms, the snails were fed ad libitum with lettuce cultivated on the unpolluted substrate described previously. Every 2 to 3 d, the microcosms were watered with 500 ml of distilled water in the plant tub, and 200 ml of distilled water were distributed homogeneously with a watering can by the top of the microcosms to simulate rainfall. During the first 12 weeks of the experiment, microcosms were in a controlled-conditions chamber with a 14:10-h light:dark photoperiod (light intensity, 150 μmol photons/m^2/s). When snails were introduced and until the end of the experiment, the photoperiod was modified to a 18:6-h light:dark photoperiod, which is better suited for snail growth [32]. During the entire experiment, the temperature was 24°C during the day and 16°C during the night. Complete randomized blocking was used for this microcosm experiment.

Sampling of organisms, preparation, and ^{137}Cs analysis

At the end of the experiments, all organisms (snails, earthworms, and lettuce leaves) were sampled. Snails and earthworms were counted and weighed to within 0.1 g to assess survival and growth, respectively. Snails and earthworms were washed with ultrapure water to remove soil particles and starved for 72 h to evacuate gut content. Then, they were killed by deep-freezing (−70°C) and stored at −20°C until drying at 60°C to constant mass. Leaves of lettuce and litter were washed to remove soil particles and dried as animals. All samples were ground.

Cesium-137 activity concentrations were measured in control organisms (i.e., unexposed snails and earthworms and lettuce grown on the unpolluted soil). For litter, replicates of litter sampled in the field, prepared in the same way as the litter introduced in containers (dried, 5 g, and same species composition), were analyzed. Most of the lettuce and snail samples were pooled by microcosm or container, because their ^{137}Cs activity concentrations were insufficient to allow individual analysis. Depending on their activity, samples were analyzed either with a standard gamma spectrometer (200 cm^3 p-type, coaxial germanium; Canberra, Lingolsheim, Alsace, France) or with a low-background one (300 cm^3 well-type germanium; Canberra) [33]. Typical detection limits for ^{137}Cs were 7 and 0.5 Bq/kg, respectively, for a 1-g sample on a 3-d measurement. The typical 661,657-keV ray of ^{137}Cs was measured, and detectors were standardized with certified solid radioactive sources containing ^{137}Cs. Counting periods varied between 12 and 72 h.

Dose rate calculation

Today, the single consensus dose rate concept for nonhuman biota is that of absorbed dose rate [18]: The activity concentration in the source of exposure is converted into energy deposited in the target per unit of time. This is obtained by

Table 2. Organisms (snail, *Cantareus aspersus*; earthworm, *Aporrectodea tuberculata*; and lettuce, *Lactuca sativa*) and media (air and soil) properties for calculations of dose rates with the EDEN (elementary dose evaluation for natural environment; Institut de Radioprotection et de Sûreté Nucléaire, Saint-Paul-Lez-Durance, France) model

Organism or medium	Density (g/cm^3)	Dimensions (mm)		
		Length	Width	Height
Earthworm	1.1	100	6	6
Snail	1.1	20	18	20
Lettuce	0.0065	300	300	400
Air	0.0012			1,000
Soil	1.7			100

multiplying the radionuclide concentration in the exposure source by an adapted coefficient, called the dose conversion coefficient, according to Equation 1 for the internal dose rate (D_{internal}):

$$D_{\text{internal}} = \sum_{\text{rn}} \text{DCC}_{\text{rn,internal}} \cdot (\text{rn, organism}) \quad (1)$$

where rn is the index corresponding to the radionuclide, $\text{DCC}_{\text{rn,internal}}$ is the dose conversion coefficient for the radionuclide rn in internal exposure (μGy/h per Bq/kg fresh mass), and [rn,organism] is the concentration of radionuclide rn in the target organism (Bq/kg fresh mass). For the external dose rate (D_{external}),

$$D_{\text{external}} = \sum_{\text{medium}} \sum_{\text{rn}} \text{DCC}_{\text{rn,external}} \cdot (\text{rn, medium}) \cdot f_{\text{medium}} \quad (2)$$

where medium is the index associated with the exposure medium, $\text{DCC}_{\text{rn,external}}$ is the dose conversion coefficient for the radionuclide rn in external exposure (μGy/h per Bq/kg fresh mass), [rn,medium] is the concentration of radionuclide rn in the considered medium (Bq/kg fresh mass), and f_{medium} is the time spent by the target organism in the exposure medium being considered. The total dose rate is then obtained by summing the external and internal dose rates (Eqn. 3):

$$D_{\text{total}} = D_{\text{internal}} + D_{\text{external}} \quad (3)$$

To determine the dose conversion coefficient according to each exposure scenario, we used a dedicated tool developed by the Institut de Radioprotection et de Sûreté Nucléaire (Saint-Paul-Lez-Durance, France), the EDEN software (Ver 2.1.2) [18]. The EDEN model runs on exposure scenarios, as defined by a combination of shapes (i.e., geometry), composition, source term (list of radionuclides of interest), and calculation options (methods and precision to apply). The geometries and densities of organisms and media are given in Table 2. Their respective chemical elemental compositions are from Beaugelin-Seiller et al. [34]. Effects of radionuclides are correlated with the energy deposited by the radiation into the target organism. The quantity and the location of this deposit result from the shapes, the composition, and the relative location of the different biological tissues and media crossed by the energy (physical calculation of particle course). The only radionuclide of interest is ^{137}Cs and its progeny in secular equilibrium, metastable barium-137 (^{137m}Ba). The branching ratio of the cesium decay is taken to be equal to 0.946; thus, the total dose conversion coefficient, or the dose conversion coefficient for the cesium family, is obtained by Equation 4:

$$D_{\text{Cs family}} = D_{^{137}\text{Cs}} + 0.946 \cdot D_{^{137m}\text{Ba}} \quad (4)$$

Table 3. Ratios of fresh mass to dry mass of the organisms (snail, *Cantareus aspersus*; earthworm, *Aporrectodea tuberculata*; and lettuce, *Lactuca sativa*) and media (air and soil) in the different treatments for calculation of dose rates with the EDEN (elementary dose evaluation for natural environment; Institut de Radioprotection et de Sûreté Nucléaire, Saint-Paul-Lez-Durance, France) model

Organism or medium (treatment)[a]	Ratio of fresh mass to dry mass	Reference
Snails (MS)	7.291	Measurement
Snails (MSP)	6.388	Measurement
Snails (MSPE)	6.476	Measurement
Earthworms (MSPE)	6.718	Measurement
Soil (all treatments)	1.000	Conservative value
Lettuce (MSP and MSPE)	19.200	[59]

[a] See Table 1 for treatment details.

The options selected for the calculations are full Monte Carlo calculation (precision, 10%) whatever the radiation type, EDEN default random seed, and because no time constraint is included, a long calculation time to gain in precision. The dose conversion coefficients evaluated are related to fresh mass of exposure source. Because the concentrations were measured on dry samples, they were corrected using adequate ratio of fresh mass to dry mass (Table 3).

Statistics and calculations

When activity concentrations were under the detection limits, we used half the detection limit value ± half the detection limit value as the mean ± standard deviation for statistical analysis. The measured variables (fresh masses of organisms and ^{137}Cs activity concentrations) were modeled with linear models, which allow significance testing of various explicative variables (absence or presence of litter and absence or presence of earthworms). When data are measured on the different individuals of one experimental unit, data should be considered as being pseudoreplicates [35]. In this case, linear mixed-effects models were used to analyze the data. These models contain both fixed (the explicative variables mentioned above) and random effects that may arise when more than one observation is measured in one experimental unit [35]. Normality of the residues was checked using a Kolmogorov–Smirnov test.

Transfer factors (TFs) of ^{137}Cs from soil to organism were calculated by dividing the ^{137}Cs activity concentrations in organisms (leaves or tissues, dry mass) by the ^{137}Cs activity concentrations in soil (dry mass).

The theoretical contribution of soil and litter in the bioaccumulation of ^{137}Cs in snails exposed in the container experiment was calculated using Equation 5:

$$\left[1 - \left(\frac{C_{t,\text{CS}} - C_{0,\text{CS}}}{C_{t,\text{CSL}} - C_{0,\text{CSL}}}\right)\right] \cdot 100 \quad (5)$$

where $C_{t,\text{CS}}$ and $C_{t,\text{CSL}}$ are the ^{137}Cs activity concentrations at time t in snails exposed in the CS and CSL treatments, respectively, and $C_{0,\text{CS}}$ and $C_{0,\text{CSL}}$ are the ^{137}Cs activity concentrations at $t = 0$ for snails exposed in the CS and CSL treatments, respectively.

The theoretical contribution of soil in the bioaccumulation of ^{137}Cs in snails exposed in the microcosm experiment was calculated using Equation 6 [31]:

Table 4. Cesium-137 (^{137}Cs) activity concentrations (Bq/kg dry mass; mean ± standard deviation) in organisms (snail, *Cantareus aspersus*; earthworm, *Aporrectodea tuberculata*; and lettuce, *Lactuca sativa*) and litter and transfer factors (TF) in container and microcosm experiments

	Snails			Earthworms			Litter		
Treatment[a]	*n*	^{137}Cs	TF	*n*	^{137}Cs	TF	*n*	Mass	^{137}Cs
Container									
Unexposed	1[b]	0.21		1[b]	0.25		4		21.80 ± 3.82
CS	4	0.60 ± 0.24	4.6×10^{-3}						
CSL	4	0.43 ± 0.19	3.3×10^{-3}				4	4.60 ± 0.37	53.21 ± 12.00
CSE	4	1.23 ± 0.21	9.4×10^{-3}	10	4.82 ± 1.96	3.7×10^{-2}			
CSLE	4	1.48 ± 0.55	11.0×10^{-3}	7	2.80 ± 1.64	2.1×10^{-2}	4	3.42 ± 0.70	78.81 ± 6.60
	Snails			Earthworms			Lettuce		
	n	^{137}Cs	TF	*n*	^{137}Cs	TF	*n*	^{137}Cs	TF
Mesocosm									
Unexposed	1[b]	0.21		1[b]	0.25		1[b]	UDL[c]	
MS	4	3.82 ± 0.25	0.03						
MSP	5	12.52 ± 2.97	0.10				5	113.94 ± 35.82	0.88
MSPE	7	15.19 ± 6.44	0.12	6	12.92 ± 4.55	0.10	6	109.23 ± 41.76	0.84

[a] See Table 1 for treatment details.
[b] One measure of a composite sample (snails, $n = 12$ individuals; earthworms and lettuce, $n = 5$ individuals).
[c] UDL = under detection limit.

$$\left[1 - \left(\frac{C_{t,\mathrm{MS}} - C_{0,\mathrm{MS}}}{C_{t,\mathrm{MSP}} - C_{0,\mathrm{MSP}}}\right)\right] \cdot 100 \qquad (6)$$

where $C_{t,\mathrm{MS}}$ and $C_{t,\mathrm{MSP}}$ are the ^{137}Cs activity concentrations at time t in snails exposed in the MS and MSP treatments, respectively, and $C_{0,\mathrm{MS}}$ and $C_{0,\mathrm{MSP}}$ are the ^{137}Cs activity concentrations at $t = 0$ in snails exposed in the MS and MSP treatments, respectively.

All statistics were performed with the R freeware (Ver 2.3.0) [36].

RESULTS

Snail and earthworm mortality and growth

In microcosms as well as in containers, no snail mortality occurred, showing that the exposure devices provided conditions supportive of snail well-being. Whatever the exposure device and treatment, the snail fresh mass increased significantly from the beginning to the end of the exposure (data not shown). In the container experiment, no significant differences in final snail fresh mass were found among treatments. In the microcosm experiment, final fresh mass of snails exposed in MS treatment (1.8 ± 0.5 g) and in MSP treatment (1.6 ± 0.3 g) were significantly higher than that of snails exposed in the MSPE treatment (1.2 ± 0.3 g). The final fresh mass of snails in the MS and the MSP treatments, however, did not differ significantly.

For earthworms, the mortality rate was 30% in microcosms. In the container experiment, mortality rates in the CSLE and CSE treatments were 0 and 20%, respectively, but these rates did not differ significantly. Fresh mass of the surviving earthworm tended to be lower at the end of the experiment than at the beginning in both containers and microcosms, but the differences also were not significant.

^{137}Cs transfer in the food web

Cesium-137 concentrations in snails, earthworms, and lettuce leaves at the end of both container and microcosm experiments were higher than those measured in nonexposed organisms (Table 4), showing a transfer of ^{137}Cs from the soil to the organisms. Average activity concentration in snails exposed in containers (regardless of treatment) was significantly lower (0.93 ± 0.54 Bq/kg dry mass) than that in snails exposed in microcosms (11.27 ± 6.77 Bq/kg dry mass).

In the container experiment (Table 4), the presence of litter did not influence the ^{137}Cs accumulation in snails. Cesium-137 accumulation in earthworm tissues tended to be lower when litter was present, but the difference was not significant. Therefore, the contribution of litter could not have been calculated either for snails or for earthworms and was considered to be negligible compared to the contribution of soil. Activity concentrations in snails were significantly increased when earthworms were present in soil. Transfer factors were of the same order of magnitude in the four exposure treatments but varied from 3.3×10^{-3} in the CSL to 11.0×10^{-3} in the CSLE treatment. Concentrations in earthworm tissues were significantly higher than those in snail tissues. Thus, the resulting TFs were one order of magnitude higher ($2.1–3.7 \times 10^{-2}$) than those of snails.

In the microcosm experiment (Table 4), ^{137}Cs concentrations in snails and earthworms did not differ in the treatment in which both organisms were present (MSPE) and were one order of magnitude higher than those found in the container experiment. The contribution of the soil in the total ^{137}Cs accumulation in snail tissues was estimated at 20%, with the contribution of lettuce thus being estimated at 80%. Activity concentrations in snails were significantly higher in the presence of *A. tuberculata*. Transfer factors for lettuce were close to one (Table 4), showing an important transfer of ^{137}Cs from soil to plant. The presence of earthworms did not significantly increase the lettuce contamination.

Effects of earthworms on soil and litter

In the container experiment, OM content at the end of the experiment was significantly higher in soils with earthworms (Table 5), but no difference was found between treatments with or without litter. The pH was significantly lower in soil containing earthworms (Table 5). Dry mass of litter in the CSL and CSLE treatments was significantly lower (8 and 32%, respectively) at the end of the experiment than at the beginning, indicating that both snails and earthworms consumed litter

Table 5. pH-H_2O and organic matter (OM) content as a function of experimental treatments at the end of the experiment[a]

Experiment	Treatment[b]	*n*	pH-H_2O	OM (%)
Containers	CS	2	6.73 ± 0.07 A	19.1 ± 0.2 A
	CSL	2	6.81 ± 0.01 A	18.9 ± 0.3 A
	CSE	2	6.15 ± 0.03 B	19.9 ± 0.4 B
	CSLE	2	6.50 ± 0.00 B	19.5 ± 0.4 B
Microcosms	MS	3	5.71 ± 0.14 A	15.8 ± 1.0 A
	MSP	3	5.79 ± 0.09 A	16.0 ± 0.7 A
	MSPE	3	5.58 ± 0.06 B	15.1 ± 0.3 A

[a] Within each group of data (pH in containers, pH in microcosms, OM in containers, and OM in microcosms), values with different uppercase letters are significantly different ($p \leq 0.05$).
[b] See Table 1 for treatment details.

(Table 4). The remaining dry mass of litter in the CSL treatment was significantly higher than that in the CSLE treatment, suggesting earthworms consumed litter in larger amounts than snails (Table 4). Moreover, ^{137}Cs activity concentrations in the litter were significantly higher at the end of the experiment for both the CSL and CSLE treatments (Table 4). This effect appeared to be higher when both organisms were present, because the litter activity concentrations were significantly higher in the CSLE than in CSL treatment (Table 4).

In the microcosm experiment, OM content did not differ significantly between soil with and soil without earthworms (Table 5). The pH-H_2O was significantly lower in the presence of earthworms (Table 5). The presence of lettuce did not influence significantly the pH.

Calculation of ^{137}Cs dose rates for snails and earthworms

The EDEN software currently is not able to consider systems as small, in terms of geometry, as the containers used in the present study. The size of the medium is automatically scaled up according to the exposure scenario, medium composition, radiation type, and the energies of the radionuclides. Therefore, dose rates were estimated for the microcosm experiment only. Based on calculated internal and external dose conversion coefficients (Table 6), total dose rates were estimated for snails for every exposure treatment and for earthworms in the MSPE treatment. Snails from the MS treatment, which were exposed to contaminated soil only, exhibited lower dose rates than those from the MSP and MSPE treatments, which were exposed to both contaminated soil and plant. For the treatment in which both snails and earthworms were present, snails exhibited higher dose rates than earthworms, which were exposed to soil only (Table 6). The contribution of lettuce to the dose rate of snails was higher than the contribution of soil (Table 6), which is in agreement with the estimate for the relative contribution of lettuce and soil in the ^{137}Cs accumulation in snail tissues. The presence of earthworms did not modify the estimate of the dose rate in snails (Table 6).

DISCUSSION

^{137}Cs transfer in the food web

A clear transfer of ^{137}Cs occurred in this soil–plant–earthworm/snail food web, showing that ^{137}Cs in this soil was bioavailable to the different organisms.

Transfer factors of up to 0.88 were calculated for lettuce in our experiments and were close to the maximal values reported in the literature for green vegetables, cabbage, and chard [37]. For earthworms, the TFs (0.10, mean) found in our microcosm experiment were low but of the same order of magnitude than those observed in other laboratory and field studies concerning radiocesium transfer from soil to earthworms. The TFs found in the literature for both ^{134}Cs and ^{137}Cs ranged from 0.03 to 1.33, depending on the earthworm species or ecological group (anecic, endogeic, and epigeic), the sources of exposure (soil, liquid medium, litter, plant leaves), the radiocesium activity concentrations, and the soil properties (OM, potassium, stable Cs or ammonium content, pH, temperature, etc). For instance, in soils spiked with ^{134}Cs at 30,000 and 50,000 Bq/kg, TFs varied from 0.2 to 0.4 for both *E. andrei* and *Lumbricus rubellus* worms (analyzed after starving) that were exposed for 14 and 21 d, respectively [38]. These TFs are higher than those found in the present study, but *E. andrei* and *L. rubellus* are epigeic species whereas *A. tuberculata* is an endogeic worm. Therefore, diet as well as digestive physiology is likely to differ, and this renders the comparison difficult. Moreover, the activity concentrations used by Janssen et al. [38] are much higher than that in our field soil, and the bioavailability of pollutants generally is much lower in field soils than in freshly spiked ones. Brown and Bell [39] exposed *Aporrectodea longa* worms to soils spiked with ^{134}Cs at

Table 6. Calculated internal and external dose conversion coefficients (DCCs) and total dose rates in snails (*Cantareus aspersus*) and earthworms (*Aporrectodea tuberculata*) as a function of experimental treatments[a]

Dosimetric parameters	Estimates and confidence indices	Snail			Earthworm
		MS	MSP	MSPE	MSPE
$DCC_{internal}$	Confidence index (%)	80	67	67	58
	Best estimate (Gy/day per Bq/kg)	3.29×10^{-9}	3.28×10^{-9}	3.28×10^{-9}	3.01×10^{-9}
	Minimum (Gy/day per Bq/kg)	3.28×10^{-9}	3.28×10^{-9}	3.28×10^{-9}	3.00×10^{-9}
	Maximum (Gy/day per Bq/kg)	3.29×10^{-9}	3.29×10^{-9}	3.29×10^{-9}	3.02×10^{-9}
$DCC_{external}$ from soil	Confidence index (%)	80	67	67	58
	Best estimate (Gy/day per Bq/kg)	1.30×10^{-9}	1.15×10^{-9}	1.15×10^{-9}	2.08×10^{-9}
	Minimum (Gy/day per Bq/kg)	1.15×10^{-9}	1.09×10^{-9}	1.09×10^{-9}	1.87×10^{-9}
	Maximum (Gy/day per Bq/kg)	1.45×10^{-9}	1.22×10^{-9}	1.22×10^{-9}	2.28×10^{-9}
$DCC_{external}$ from lettuce	Best estimate (Gy/day per Bq/kg)	No lettuce in this treatment	3.13×10^{-9}	3.13×10^{-9}	Not calculable
	Minimum (Gy/day per Bq/kg)		2.65×10^{-9}	2.65×10^{-9}	
	Maximum (Gy/day per Bq/kg)		3.62×10^{-9}	3.62×10^{-9}	
Total dose rate	Best estimate (μGy/h)	7.56×10^{-3}	22.8×10^{-3}	22.5×10^{-3}	12.9×10^{-3}
	Minimum (μGy/h)	6.75×10^{-3}	20.2×10^{-3}	20.0×10^{-3}	11.8×10^{-3}
	Maximum (μGy/h)	8.38×10^{-3}	25.5×10^{-3}	25.1×10^{-3}	14.0×10^{-3}

[a] See Table 1 for treatment details.

302,000 Bq/kg and to a litter made of apple leaves spiked with ^{137}Cs at 3,629,000 Bq/kg. Transfer factors (calculated using worms without gut content, as in the present study) ranged from 0.034 to 0.052, depending on the exposure duration (from 18 to 24 d) and on the exposure source (soil or leaves). *Aporrectodea longa* and *A. tuberculata* are two endogeic species belonging to the same genus, so the comparison of those TF values with our data probably is more valid. The values found by Brown and Bell [39] are much lower than those in the present study. This could be a result of the much higher exposure concentrations, because transfer parameters generally decrease with increasing concentrations in the environment. In their study of radionuclide transfers in coniferous forests in the vicinity of a reprocessing plant in the United Kingdom, Copplestone et al. [40] found ^{137}Cs activity concentrations ranging from 478 to 620 Bq/kg in undetermined Oligochaeta individuals (analyzed without starving) and calculated TFs of 0.90 and 1.33. Such high values could have been increased by the presence of soil in the worm gut, but high values also were found in starved individuals sampled in the field. Indeed, in two woodland areas of northern France, *L. rubellus* worms exhibited TFs of 0.17 and 0.94 in soils of the Jura and Vosges mountains, respectively [41]. Globally speaking, epigeic species in both laboratory and field studies seem to exhibit higher TFs than endogeic worms, possibly because upper layers usually are more contaminated than deeper soil and because of different ecological or physiological features.

The TFs found in snails exposed in the container experiment (ranging from 0.004 in the absence of earthworms to 0.011 in the presence of worms) were lower than those found in individuals sampled in contaminated areas. Gaso et al. [20,42] found TFs ranging from 0.017 to 0.184 in *C. aspersus* snails living in the surroundings of a radioactive waste site in central Mexico, where ^{137}Cs activity concentrations in soils ranged from 26 to 181 Bq/kg dry mass (whether animals were starved was not specified) [20,42]. Kalinowska and Grzybowska [43] found TFs ranging from 0.01 (shell) to 0.12 (body) in the land snail *Succinea putris* living in the surroundings of a nuclear power plant in Poland, where activity concentrations in soils ranged from 5.9 to 40 Bq/kg dry mass (no precision on starving of animals was given). The low TFs found in snails exposed in containers could result from the absence of contaminated plant and a low duration of exposure. For the microcosm experiments in which snails were exposed to more realistic conditions (longer exposure duration and contaminated plants), TFs calculated for snails reached 0.12 and were more in agreement with those found in field sites [20,42].

In the microcosm experiment, ^{137}Cs activity concentrations in both snails and earthworms were higher than those in the container experiment, probably because of the longer exposure duration. Further comparison between the two experimental designs showed that concentrations in earthworms and in snails were similar in the microcosm experiment, whereas they were 10-fold higher in earthworms than in snails in the container experiment. This could result mainly from the different exposure sources for snails in the two studied designs (containers vs microcosms). Snails were, indeed, exposed to contaminated soil only in containers, whereas they were exposed to both contaminated soil and lettuce in microcosms. The transfer was maximal from soil to plant, and the resulting ^{137}Cs activity concentrations in the lettuce were an order of magnitude higher than those measured in the invertebrates. For the different organisms in the present study, the TFs ranged from 0.004 to 0.88, showing that biomagnification did not occur in this food web.

Contribution by sources to ^{137}Cs accumulation in animals

The majority contribution of lettuce (80%) in the total accumulation of ^{137}Cs in snail tissues is in agreement with literature data showing that ^{137}Cs accumulation in vertebrates is mainly the result of contaminated foodstuffs, even if soil can be an additional source [44,45]. The high contribution of lettuce in snail contamination could be explained by a majority quantity of lettuce in the snail diet and/or by a higher bioavailability of ^{137}Cs in plant tissues than in soil. This last hypothesis is supported by two studies showing that the bioavailability of Cs incorporated in vegetation was higher than that of soil-associated radiocesium. In the sheep, bioavailability of soil-associated radiocesium was approximately 20% of that when the activity was incorporated in vegetation via root uptake [46]. For earthworms, assimilation of cesium from leaves also has been shown to be higher than that from soil [39]. Our experiments, however, do not provide accurate data supporting or refuting one or the other of those two possible explanations (difference of diet or of bioavailability), which of course are not exclusive.

In previous work using the same microcosms and controlled conditions as in the present study, the relative contribution of plants (lettuce) and soil in the accumulation of metals by *C. aspersus* snails was shown to vary, depending on the studied metal [31]. Those authors suggested that for an exposure duration of two to eight weeks, the contribution of soil in the accumulation of lead in snail tissues was relatively constant and high (84–89%). The low contribution of plant resulted from a low transfer of lead from the soil to the lettuce. The soil contribution varied from 63 to 32% for zinc, which is closer than what we found for ^{137}Cs, and varied from 2 to 40% for cadmium, preventing clear interpretation [31].

Effect of earthworms on ^{137}Cs transfer

In the present study, earthworms slightly but significantly increased the transfer of ^{137}Cs from soil to snails. This is in agreement with previous results showing an increased transfer of cadmium, copper, and zinc from soil to *C. aspersus* snails when *A. tuberculata* earthworms were present in the soil [14]. In this latter experiment, the exposure design of which is close to our container design, internal cadmium, copper, and zinc concentrations in snails were increased by a factor of 1.66, 1.46, and 1.30, respectively. In the present study, ^{137}Cs activity concentrations in snails were increased by a factor of 2.1 to 3.4 in containers and by a factor of 1.2 in microcosms. Coeurdassier et al. [14] proposed two nonexclusive hypotheses to explain this phenomenon. First, earthworms may increase the availability of the contaminant, as suggested by their results for cadmium (i.e., the water-soluble cadmium fraction was increased in the soil with earthworms, and this increase was accompanied by a higher cadmium concentration in snail tissues) [14]. We cannot bring new elements to support this hypothesis, because we did not measure the availability of ^{137}Cs. If this effect had occurred at the whole-soil scale, however, one could hypothesize that the increase in the availability of ^{137}Cs would have led to a higher activity concentration in the lettuce, which was not observed in the present study. The second hypothesis relies on a possible effect of earthworms on the soil structure. Coeurdassier et al. [14] argued that metallic trace element concentrations in the casts can be higher

than those in the surrounding soil [47] and that because surface horizons of numerous soils are mainly made of casts produced by earthworms [9], these organisms may increase the contamination of the soil surface, which is the most accessible to snails. Earthworms may have increased the availability of radiocesium in their casts deposited at the soil surface, thus increasing the exposure of snails but not of the plant. In the case of ^{137}Cs, earthworm casts had higher concentrations of OM and available mineral nutrients, such as potassium, whose ^{137}Cs is an analogue, compared with the surrounding soil [39,48]. The underlying mechanisms suggested to be involved in this increased availability are the digestive processes in earthworms [49] and the structure and composition of their casts (important organic content, high moisture, and increased microbial activity) [39]. Analysis of Cs availability by chemical extraction in the casts and in the upper and deeper soil layers would be needed to improve the understanding of those mechanisms.

In both container and microcosm experiments, earthworm activity significantly decreased the soil pH. From a literature review, Avery [1] noted that solubility and mobility of ^{137}Cs might increase with decreasing pH, because ^{137}Cs ions bound to clay particles might be replaced by H^+. Other authors, however, have suggested that the soil pH itself may be of minor importance but that the factors influencing pH could be responsible for changes in Cs^+ availability [1]. Frissel et al. [50] indicated that plant TFs seemed to increase as a result of pH only if the soil pH drops below 4 to 5. Therefore, whereas the effect of earthworms on pH might not have been large enough to influence radiocesium phytoavailability, their cast production may have been sufficient to increase ^{137}Cs transfer from soil to snail. To date, results concerning the possible effects of earthworms on phytoavailability are contradictory. Liu et al. [13] reported that cadmium and copper concentrations were significantly lower in cabbage cultivated on sewage sludge (spiked with a mixture of cadmium and copper) when *E. fetida* earthworms were present. In another experiment, the activity of *E. fetida* earthworms has been shown to increase the transfer of copper, chromium, and zinc from five field (nonspiked) soils to wheat (*T. aestivum*) [12]. Wen et al. also showed that the concentrations of five rare earth elements were increased in wheat grown on soils containing *E. fetida* [51]. Other authors have suggested that the bioavailability of heavy metals could be modified by earthworms on the basis of an increase in metal availability (assessed by various chemical extractions) but have not experimentally tested this hypothesis [52,53].

Effect of ^{137}Cs on mortality, growth, and dose rate

No effect either on mortality or on growth was observed in snails and worms in these experiments, which is consistent with the low dose rate values estimated with the EDEN software. The lowest dose rate reported in the literature as a protective value for terrestrial animals is 1 mGy/d (~42 μGy/h [54]). A more traceable value of 67 μGy/h was established within the Environmental Risk from Ionizing Contaminants: Assessment and Management project [55]. This value corresponds to the hazardous dose rate affecting 5% of species with 50% confidence issued from the species-sensitivity distributions built on the dose rate leading to 10% of observed effect for terrestrial organisms. From this value, 10 μGy/h generally is used in risk assessment. This value is deduced from the hazardous dose rate affecting 5% of species with a 50% confidence value of 67 μGy/h by applying a safety factor of five rounded down to the nearest decimal (67/5 = 13.4 rounded to 10). Whatever the limit value considered, the dose rates calculated for the animals in the microcosms are at least three orders of magnitude lower. In terms of risk (using the quotient method), such dose rates lead to a maximal risk of 3.0×10^{-4}, which is insignificant.

Some limitations apply to this conclusion. First, the limit values reported here concern the whole terrestrial ecosystem for a selection of endpoints. Several kinds of effects, mainly cytogenetic ones, however, have been reported for aquatic and terrestrial fauna and flora at lower dose levels (between 0 and 50 μGy/h [Frederica database, http://www.frederica-online.org]), but their relevance on the long-term health of these organisms has not been established. Moreover, no specific dose–effects data are available for earthworms and snails. Second, the dose conversion coefficients used were not weighted to integrate the relative biological effectiveness of the different types of radiation, which is especially significant for the alpha emitters. In the present case, this omission is of minor effect, because no alpha contribution is found in the ^{137}Cs radiation. Finally, the main restrictions to the validity of the dose rate calculation are those linked to the necessary simplifications and assumptions underlining the EDEN code, the validation of which has been discussed elsewhere [56].

According to our results, the presence of *A. tuberculata* earthworms in a soil increased the transfer of ^{137}Cs from soil to another soil organism, the snail *C. aspersus*, but had no detectable effect on ^{137}Cs phytoavailability. The mechanisms by which earthworms modify ^{137}Cs (bio)availability are still poorly known and require further research. The presence of contaminated litter collected from the same site as the contaminated soil did not influence the transfer of ^{137}Cs in our experiments. Studies concerning radiocesium behavior in the natural environment have demonstrated that mechanisms involved with its transfer in natural ecosystems are numerous and complex and that our knowledge of these mechanisms must be improved [6,57,58]. In the present study, we have shown that biotic interactions like earthworm activity, which have scarcely been studied by comparison with physicochemical parameters, should attract more attention to better understand the mechanisms involved in bioavailability and transfer of radiocesium. We conclude that such biotic interactions and other concepts and processes linked to communities should be considered more for contaminant transfer modeling and risk assessment.

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