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Marking earthworms for release–recapture studies using the trace element rubidium

Mostafa Ben Hamou [a], *Aleš Kulhánek* [b], *Simon Lacombe* [c], *Joann K. Whalen* [a,*]

[a] *Department of Natural Resource Sciences, Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, Québec, Canada*
[b] *Department of Environmental Chemistry, Institute of Chemical Technology, Prague, Czech Republic*
[c] *Département de biologie, Centre de Recherche en Biologie Forestière, Université de Sherbrooke, Sherbrooke, Québec, Canada*

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ABSTRACT

A classic method to assess animal populations is to mark a population, release them into the wild, and make measurements on individuals that are captured after a period of time. The objective of this study was to determine whether earthworms can assimilate and retain sufficient rubidium (Rb) in their tissues to differentiate marked and unmarked earthworms. Fifty adult and 50 juvenile earthworms (*Aporrectodea turgida* (Eisen)) were placed in individual pots with soil containing 500 mg Rb kg^{-1} for 1 week. Earthworms assimilated Rb at rates of 23–26 μg Rb g^{-1} earthworm fresh weight day^{-1}, and the Rb concentration in earthworm tissue was 100-fold greater than in unmarked earthworms after 1 week. When we transferred marked earthworms to clean soil, they eliminated about 50% of the Rb in their tissues within 3 days. The Rb concentration declined exponentially during the elimination period, but remained 10 times greater in marked earthworms (78.6–112.4 μg Rb g^{-1} oven-dry tissue) than unmarked earthworms (on average, 5.7 μg Rb g^{-1} tissue). These results indicate that marking earthworms with Rb may be an effective way to track individuals and differentiate marked earthworms from indigenous populations in ecological release–recapture studies.

1. Introduction

Direct observation at the soil surface of earthworms and their structures (burrow entrances, casts, middens) provides information on the mobility and spatial distribution of some earthworm species. Direct observation provides less information about cryptic species, such as the endogeic earthworms that inhabit the mineral soil layers (Edwards and Bohlen, 1996). Mark–recapture techniques may be more effective to monitor the mobility, distribution and behavior of these earthworms in their natural environment. Yet, classical markers such as paint and tattoos do not persist due to continual mucus secretion and abrasion as earthworms burrow through the soil, and tagging or physically mutilating these small soft-bodied organisms is often fatal (Eriksen-Hamel et al., personal communication). Therefore, a chemical marker that is incorporated and retained in earthworm tissue may be more suitable. The element selected should persist in measurable concentrations for a sufficient period of time (i.e., several weeks or months), without affecting earthworm biological functioning. It should also be safe for release into the environment.

Many researchers have already demonstrated that earthworms can be labeled with the stable isotopes ^{13}C and ^{15}N, but the technique has not been used for mark–recapture studies due to the cost of stable isotope analyses (Barois et al., 1987; Cortez et al., 1989; Schmidt et al., 1999; Whalen and Janzen, 2002). Radioactive carbon and cesium are also incorporated

* *Corresponding author at*: Department of Natural Resource Sciences, Macdonald Campus of McGill University, 21, 111 Lakeshore Road, Ste-Anne-de-Bellevue, Québec, Canada H9X 3V9. Tel.: +1 514 398 7943; fax: +1 514 398 7990.
E-mail address: joann.whalen@mcgill.ca (J.K. Whalen).
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readily and retained in earthworm tissue (Crossley et al., 1971; Cortez et al., 1989; Brown and Bell, 1995), but might pose a risk of environmental contamination. Other non-radioactive trace elements, such as lithium and rubidium, have been used as markers for release–recapture studies with insects (Dombos and Stimmann, 2001; Hagler and Jackson, 2001), but have not yet been investigated for earthworms.

The objective of this study was to determine whether earthworms can assimilate and retain sufficient Rb in their tissues to tissues to differentiate marked and unmarked earthworms.

2. Materials and methods

2.1. Earthworms and soil collection

Earthworms and soil were collected from the Macdonald Research Farm, Ste-Anne-de-Bellevue, Quebec, Canada (45°28′N, 73°45′W). Earthworms were collected in October 2004 by hand-sorting the soil from a field under soybean (*Glycine max* (L.) Merrill) production. Sexually mature individuals were identified as *Aporrectodea turgida* (Eisen) based on the Schwert (1990). Earthworms were maintained in cultures containing soil moistened to 20% gravimetric moisture content at 20 °C for 2 weeks before the experiment began. Soil used in this study was a sandy-loam mixed, frigid Typic Endoquent of the Chicot series containing 58% sand, 30% silt and 12% clay, with 30.2 g organic C kg^{-1} and a pH (H_2O) of 6.3.

2.2. Experimental design

About 40 g of soil (air dry, sieved <2 mm) was placed in a 120 cm^3 plastic cup (5 cm diameter, 6 cm height) and moistened to 16% gravimetric moisture content with either distilled water (control soil) or a 2.5 g Rb L^{-1} solution (Rb-labeled soil). The Rb-labeled soil contained 500 μg Rb g^{-1} from RbCl (Fisher Chemicals, Fairlawn, NJ, USA). We prepared 60 cups of control soil and 100 cups of Rb-labeled soil. Soils were incubated in the dark for 4 days at 20 °C. Then, 1.6 mL of 10% dextrose solution was applied to all soils to bring the moisture content to 20% (gravimetric basis) and stimulate microbial activity, and incubated for an additional 3 days at 20 °C before adding earthworms.

Individuals of *A. turgida* were rinsed and placed on wet filter paper to void their guts for 24 h. The next day, earthworms were rinsed, gently blotted dry with paper towels and weighed (gut-free fresh weight). We selected healthy earthworms (80 juveniles and 80 clitellate adults) and placed them cups with control soil (30 individuals from each age class) or Rb-labeled soil (50 individuals of each age class). We moistened the earthworm and soil surface with 1–2 mL of distilled water, covered the cups with perforated lids to prevent earthworm escape and placed them in the dark at 20 °C.

After 1 week, earthworms were removed from all cups, placed on wet filter paper to void their guts for 24 h and then weighed (gut-free fresh weight). Earthworm weight gain was calculated as: (earthworm final weight – earthworm initial weight)/earthworm initial weight (iw) and expressed on a gram/gram iw basis. Ten juveniles and 10 adults from each treatment (control soil, Rb-labeled soil) were euthanized by immersion in boiling water for 5 s. They were oven-dried at 60 °C for 48 h and then ground finely with a mortar and pestle for Rb analysis. Samples of the control soil ($n = 5$) and the Rb-labeled soil ($n = 5$) were also oven-dried (60 °C for 48 h), finely ground and analyzed to verify the soil Rb concentration.

The other earthworms were placed in 120 cm^3 cups containing 40 g (dry weight basis) of unlabeled soil (prepared like the control soil, described above). Cups were covered with perforated lids and placed in the dark at 20 °C. The elimination of Rb from earthworm tissue was assessed by removing juvenile and adults of *A. turgida* from unlabeled soil after 3, 9, 19 and 39 days. At each sampling date, juveniles and adults from the control soil ($n = 5$ for each age class) and Rb-labeled soil ($n = 10$ for each age class) treatments were selected at random. After their gut-free fresh weight was determined, earthworms were euthanized in boiling water, oven-dried (60 °C for 48 h) and ground for Rb analysis.

2.3. Rubidium analyses

We followed the procedure of Dombos and Stimmann (2001), with some modifications. Oven-dried earthworm tissue (30–120 mg) and soil (150 mg) samples were placed in 100 mL digestion tubes with 5 mL of concentrated HNO_3 (trace metal grade) overnight (about 14 h), then digested at 140 °C for 1 h. After cooling, one mL of 30% H_2O_2 solution was added to each tube, the samples were digested at 140 °C for 30 min, and this step (cooling, adding 1 mL of 30% H_2O_2) was repeated until a clear solution was obtained. Samples were diluted to 15 and 0.1 mL of $CsCl_2$ was added to stabilize signal detection, before the Rb concentration was determined at 780 nm on a Perkin-Elmer 2380 Atomic Absorption Spectrophotometer (Perkin-Elmer, Wellesley, MA, USA). We report the Rb concentration in earthworms as μg g^{-1} oven-dry tissue. The quantity of Rb assimilated by earthworms was the difference in the tissue Rb concentration of individuals from the Rb-labeled soil and the control soil.

2.4. Statistical analyses

The effects of the Rb treatment on earthworm weight and Rb concentration in tissue of juvenile and adult earthworms were evaluated with pairwise Student's *t*-tests (95% confidence level). The functional relationship between Rb concentrations and initial earthworm biomass was fitted using structural analysis (Webster, 1997), whereas the rate of Rb elimination from earthworm tissue during a 39 days period was described using an inverse function.

3. Results

3.1. Assimilation of Rb by A. turgida

Juveniles and adults exposed to the Rb-labeled soil had significantly ($P < 0.05$) more Rb in their tissues than those in the control soil (Table 1). Three individuals (two juveniles and one adult) from the control soil treatment died during

Table 1 – Weight gain (g g^{-1} earthworm initial weight (iw)) and Rb concentration (μg Rb g^{-1} oven-dry tissue) in juvenile and adult *A. turgida* after feeding on Rb-labeled or control soil for 1 week

Age class	Soil	Weight gain (g g^{-1} iw)	*P* value	Rb concentration (μg Rb g^{-1})	*P* value
Juvenile	Rb-labeled	0.15 ± 0.05	0.98	913.2 ± 62.0	<0.0001
	Control	0.17 ± 0.04		9.1 ± 2.6	
Adult	Rb-labeled	0.07 ± 0.04	0.91	816.1 ± 38.8	<0.0001
	Control	0.08 ± 0.02		7.5 ± 1.1	

Values are the means (±S.E.) of 10 replicates, with *P* values calculated for each age class (Student's *t*-test).

the study, but there was no mortality in the Rb-labeled soil treatment. Earthworms gained weight during the study, but there was no difference in the standard weight gain of juveniles and adults from the control and Rb-labeled soil treatments (Table 1). The mean quantity of Rb assimilated by adults was 808.6 μg Rb g^{-1} oven-dry tissue, indicating a Rb uptake rate of about 23.1 μg Rb g^{-1} earthworm fresh weight day^{-1} if we assumed that earthworm dry weight was 20% of earthworm fresh weight (Edwards and Bohlen, 1996). The average Rb uptake rate for juveniles was 25.8 μg Rb g^{-1} earthworm fresh weight day^{-1}. 1. We noted that Rb accumulation in earthworm tissue was correlated negatively with the initial earthworm fresh weight (Fig. 1).

3.2. *Elimination of Rb from A. turgida*

The elimination of Rb from *A. turgida* was fitted with an inverse (1/*x*) function, and half of the Rb initially present in earthworm tissue was eliminated after 1–3 days when individuals were placed in unlabeled soil (Fig. 2). By the end of the 39 days post-labeling period, the Rb concentration in juveniles exposed to the Rb-labeled soil was 112.4 μg Rb g^{-1} oven-dry tissue, while adults contained 78.6 μg Rb g^{-1} oven-dry tissue. This was more than 10-fold greater than the Rb concentration of earthworms in the control soil, which averaged 7.6 ± 1.0 μg Rb g^{-1} ($n = 20$) in juveniles and 2.3 ± 0.9 μg Rb g^{-1} ($n = 20$) in adults.

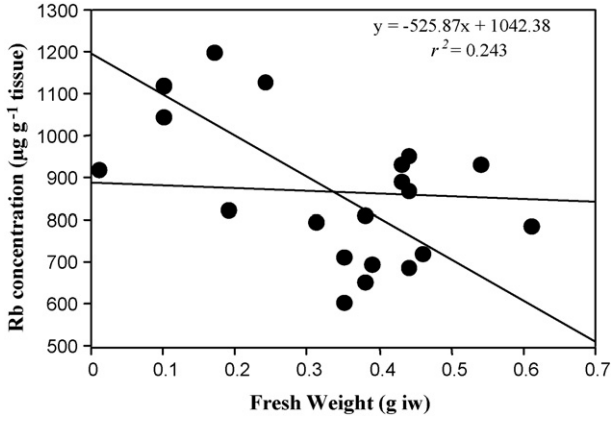

Fig. 1 – Functional relationship between earthworm initial weight (g iw) and the Rb concentration in earthworm tissue ($n = 20$). The solid line represents the linear regression, with dashed lines indicating the 95% confidence interval.

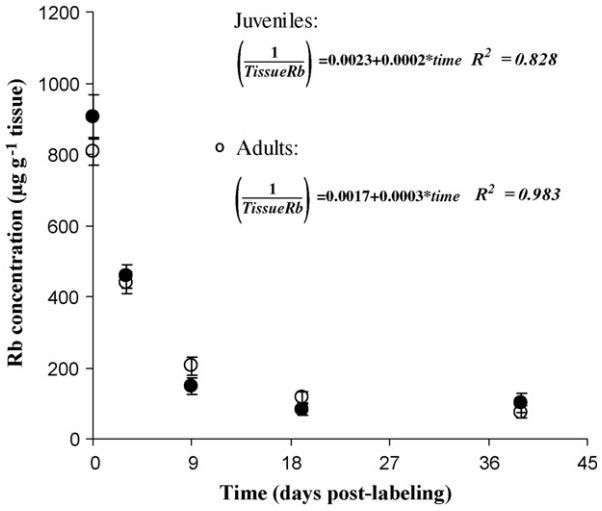

Fig. 2 – Elimination of Rb from the tissue of juvenile and adult *A. turgida* during the 39 days post-labeling period. Equations describing Rb elimination rates are provided for each earthworm age class. Each point is the mean (with standard error bars) of 10 replicates.

4. Discussion

The trace element Rb has been used as a marker for plants, soil microorganisms, collembola, plant parasites and insects (Berry et al., 1972; Graham et al., 1978; Johnson and Reeves, 1995; Dombos and Stimmann, 2001; Hagler and Jackson, 2001). As far as we know, this is the first report of the use of Rb as a physiological marker for earthworms. The use of Rb as a marker provides some advantages over other elements. First, it is an environmentally safe metal (not radioactive) and marked samples are easily detected because the natural background levels are low, generally not more than 5–10 ppm Rb (Lide, 1998). Moreover, there is vertical transmission of Rb between invertebrate trophic levels, and concentrations decrease with each trophic ascension (Graham et al., 1978; Johnson and Reeves, 1995; Corbett et al., 1996). These characteristics make Rb a suitable marker for field studies (Stimmann, 1991). In addition, the analysis of Rb is expected to be inexpensive, relative to other markers, because it requires a one-time purchase of a lamp for an atomic absorption spectrometer, plus normal operating costs (perhaps $2–3 per sample). In contrast, stable isotope (^{13}C, ^{15}N) analysis with mass spectrometry costs $5–10 per sample, while radioisotope (^{14}C, ^{55}Cs) analysis may be even more expensive because it

requires specialized laboratory facilities and training to protect laboratory personnel.

The Rb marker was assimilated at rates of 23–26 μg Rb g^{-1} earthworm fresh weight day^{-1} by *A. turgida*, and Rb concentrations were more than 100 times greater in marked than unmarked earthworms after 1 week. Earthworm survival and weight gains were similar when individuals were exposed to Rb-labeled soil and the control soil, suggesting no toxic effects. Because its chemical properties are similar to those of potassium, Rb is assimilated at low and moderate levels in biological systems without deleterious effects (Stimmann, 1974; Graham and Wolfenbarger, 1977; Knight et al., 1989; Kipp and Lonergan, 1992). However, exposure to high Rb concentrations has led to sub-lethal toxicity in some insect taxa (Van Steenwyk et al., 1978; Cullin and Alverson, 1986; Knight et al., 1989). Further work is needed to confirm the optimal Rb dose and exposure time for various earthworm species.

The uptake of Rb was inversely proportional to the biomass of individuals, and larger earthworms contained less Rb in their tissues than smaller individuals. Similar relationships have been reported for earthworms marked with ^{13}C and ^{15}N stable isotopes (Whalen and Janzen, 2002; Dyckmans et al., 2005). Greater quantities of biological markers may be incorporated into the tissues of young, fast-growing individuals than in earthworms that have reached reproductive maturity. However, the pattern of Rb elimination was similar in both juvenile and adult *A. turgida*. The rapid elimination of Rb in the first 1–3 days of the post-labeling period, followed by a slower rate of Rb elimination, is similar to the exponential loss of other elements from earthworm tissue (Crossley et al., 1971; Barois et al., 1987; Brown and Bell, 1995). It seems likely that some of the Rb in earthworms was replaced by K, its chemical analog, when earthworms consumed unlabeled soil. This mechanism has been suggested to explain the elimination of Rb from plants and arthropods (Thoeny et al., 1992; Dombos and Stimmann, 2001). In addition, earthworms still retained a small amount of soil in the gut even after the 24 h gut clearance (Whalen and Janzen, 2002). This could lead to a slight overestimation of the Rb concentration in earthworms after the 1 week labeling period, since some Rb-labeled soil may have been included with the earthworm tissue, and a slight underestimation of the Rb concentration in earthworms during the post-labeling period, since the presence of unlabeled soil would dilute the Rb concentration in earthworm tissues. Although dissections to remove the earthworm gut would provide more accurate measurements of the Rb concentration in earthworm tissue, this would be prohibitively time-consuming when large numbers of earthworms are collected. For ecological studies aiming to monitor earthworm survival and migration within the soil profile or across landscapes, it may be enough to know how many of the marked individuals released into the environment were recaptured.

Our key findings were that Rb was assimilated rapidly by earthworms without obvious deleterious effects, and that marked earthworms were distinguishable from umarked earthworms more than a month after they were exposed to Rb. This new tool may be used by researchers wishing to track and distinguish introduced earthworms from indigenous field populations. Our ongoing research (i.e., Eriksen-Hamel and Whalen, 2006) attempts to quantify the contribution of earthworms to N cycling and crop production in enclosures with known earthworm populations (naturally occurring populations are reduced by applying carbaryl pesticide repeatedly, and specific numbers/species of earthworms are added). We do not know whether introduced earthworms have the same behavior (feeding and burrowing activities), reproduction and survival rates as indigenous earthworms, and we cannot distinguish introduced earthworms from indigenous earthworms that persisted after pesticide applications. Being able to differentiate marked and unmarked earthworms would improve greatly our interpretation of data from such field studies. manipulation studies.

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