



Published in final edited form as:

*Soil Biol Biochem.* 2015 June 1; 85: 190–198. doi:10.1016/j.soilbio.2015.03.001.

## Trace Metals and Metalloids in Forest Soils and Exotic Earthworms in Northern New England, USA

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### Abstract

Trace metals and metalloids (TMM) in forest soils and invasive earthworms were studied at 9 uncontaminated sites in northern New England, USA. Essential (Cu, Mo, Ni, Zn, Se) and toxic (As, Cd, Pb, Hg and U) TMM concentrations ( $\text{mg kg}^{-1}$ ) and pools ( $\text{mg m}^{-2}$ ) were quantified for organic horizons (forest floor), mineral soils and earthworm tissues. Essential TMM tissue concentrations were greatest for mineral soil-feeding earthworm *Octolasion cyaneum*. Toxic TMM tissue concentrations were highest for organic horizon-feeding earthworms *Dendobaena octaedra*, *Aporrectodea rosea* and *Amyntas agrestis*. Most earthworm species had attained tissue concentrations of Pb, Hg and Se potentially hazardous to predators. Bioaccumulation factors were  $\text{Cd} > \text{Se} > \text{Hg} > \text{Zn} > \text{Pb} > \text{U} > 1.0 > \text{Cu} > \text{As} > \text{Mo} > \text{Ni}$ . Only Cd, Se Hg and Zn were considered strongly bioaccumulated by earthworms because their average bioaccumulation factors were significantly greater than 1.0. Differences in bioaccumulation did not appear to be caused by soil concentrations as earthworm TMM tissue concentrations were poorly correlated with TMM soil concentrations. Instead, TMM bioaccumulation appears to be species and site dependent. The invasive *Amyntas agrestis* had the greatest tissue TMM pools, due to its large body mass and high abundance at our stands. We observed that TMM tissue pools in earthworms were comparable or exceeded organic horizon TMM pools; earthworm tissue pools of Cd were up 12 times greater than in the organic horizon. Thus, exotic earthworms may represent an unaccounted portion and flux of TMM in forests of the northeastern US. Our results highlight the importance of earthworms in TMM cycling in northern forests and warrant more research into their impact across the region.

### Keywords

mercury; lead; selenium; invasive earthworms; heavy metals; trace elements; bioaccumulation; unpolluted soil

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## 1. Introduction

Forest soils contain large terrestrial pools of trace metals and metalloids (TMM) that potentially can be taken up by terrestrial organisms, leached into groundwater, or transferred to surface waters. Many of the TMM have been enriched through atmospheric deposition from human sources with varying implications for humans and wildlife (Steinnes and Friedland, 2006; Kaste et al., 2006; Driscoll et al., 2007; Juillerat et al., 2012; Richardson et al., 2013; Richardson et al., *in press*). Essential TMM, such as Cu, Mo, Ni, Se and Zn, are required by plants and animals as enzyme cofactors and micronutrients (Adriano 2001; Alloway 2013). Toxic TMM, such as As, Cd, Pb, Hg and U, are not required by organisms and may cause acute or chronic toxicity in plants, microorganisms, wildlife and humans (Friedland et al. 1986; Adriano, 2001; Sparks 2003; Steinnes and Friedland, 2006). Understanding the fate and transport of TMM in non-contaminated forest soils is imperative for predicting burdens to aquatic and terrestrial ecosystems and soil fertility (Greig-Smith et al., 1992; Adriano 2001; Driscoll et al. 2007).

Multiple exotic species of earthworms from Europe and Asia are now found in the northern forests of Vermont and New Hampshire as a result of human activities, particularly fishing, logging, waste management and horticulture (Bohlen et al. 2004; Frelich et al. 2006; Görres and Melnichuk, 2012). These earthworms can alter the morphology and elemental cycling within forest soils by consuming the organic horizon (forest floor), mixing soil horizons, and increasing mineralization of soil organic matter (SOM) (Edwards and Bohlen, 1996; Bohlen et al. 2004; Frelich et al 2006). These actions modify C, N and P cycling in the forest soils of New England by destroying soil aggregates and decreasing % SOM concentrations, both of which promote TMM retention (Edwards and Bohlen, 1996; Adriano, 2001; Suárez et al., 2003, Bohlen et al. 2004; Frelich et al 2006; Steinnes and Friedland, 2006; Sizmur and Hodson, 2009; Fahey et al. 2013). In aggregate, their actions may result in a loss of essential metals required by northern hardwood forests (Bohlen et al., 2004; Frelich et al., 2006; Alban and Berry, 1994, St Clair et al. 2008; Resner et al., 2014) and potentially cause remobilization and subsequent leaching of TMM that have accumulated in the organic horizon and upper mineral soil horizons from multiple decades of enhanced atmospheric deposition (Steinnes and Friedland, 2006; Driscoll et al., 2007).

Most studies on TMM in earthworms have focused on bioaccumulation at contaminated stands from point source or amendments such as sewage (e.g. Ireland, 1979; Dai et al. 2006; Suthar et al., 2008; Zhang et al., 2009), often elevating soil concentrations of Cu, Ni, Zn, Cd, Pb, and Hg beyond ranges observed in forests of the eastern United States and Canada (Evans et al., 2005; Landre et al., 2010; Richardson et al., 2015). However, the bioaccumulation of toxic TMM such as Cd, Pb and Hg at non-contaminated stands is of concern because they may attain tissue concentrations hazardous to a number of their terrestrial macrofaunal predators (Grieg-Smith et al., 1992; Rieder et al., 2011). This study presents concentrations and pools (mass per unit area) of TMM in soils and exotic earthworms in northern New England, USA, at remote forested sites away from known point sources of pollution. The objectives of this study were: 1) quantify the concentrations and pools of TMM in forest soils and exotic earthworms in northern New England; 2) assess if there are particular soil and earthworm properties that are associated with TMM

accumulation in earthworms. This information is necessary to informing a variety of environmental scientists, forest managers and policy makers on a potential fate of TMM in forest soils that are adjacent to the headwaters of important northeastern water resources.

## 2. Methods

### 2.1 Site Descriptions

In total, 45 forested stands at 9 sites with varying soil properties were studied in northern Vermont and New Hampshire (Table 1). All stands were located on privately owned and managed land. Sites 1 – 6 were chosen from the 2003 Regional Environmental Monitoring and Assessment Program (Kamman et al., 2003), for their extensive existing watershed information. Sites 7 – 9 were located in lowland forests with known points of earthworm introduction by humans. Stands were randomly selected from areas that were well-drained, on gentle slopes ( $< 10^\circ$ ) at least 50 m away from any human development, and at least 50 m away from each other. Geomorphic positions sampled were only toe slopes and terraces. Earthworm middens, mounds and other forms of disturbance were not used to determine stand location. Each stand was considered an independent sample of each site because of the  $> 50$  m distance among them and random because visible signs of earthworms were not used. Sites 1, 5, and 7 were considered remote, due to no residential developments within 1 km and no trails to sampling locations. All other stands have residential developments within 1 km and are within 100 m of a road. Forest stands were generally uneven-aged and composed of deciduous vegetation: *Acer saccharum*, *Betula papyrifera*, *Betula alleghaniensis*, and *Fagus grandifolia* and coniferous vegetation (*Picea* spp. *Pinus* spp., and *Tsuga canadensis*) (Table 1). All soils were acidic ( $\text{pH} < 5$ ) and primarily Inceptisols. The mapped soil series for each stand according to the Web Soil Survey (2013) and confirmed by field observation. A detailed set of information for each stand is given in Supplemental Table 1.

### 2.2 Sample collection and preparation

Stands were sampled in September and October, 2013. At each stand, one morphological pit was excavated to describe soil horizons. Soil profiles in the morphological pit were described following the USDA taxonomic guide (Soil Survey Staff, 2010). In addition, one quantitative soil pit was excavated by horizon to estimate TMM soil concentrations, total horizon mass and total earthworm densities, respectively. Organic horizon (forest floor) samples were collected using a 15 cm  $\times$  15 cm template. Organic horizon samples generally consisted of the Oi, Oe and Oa horizons, with Oe and Oa absent at some sites. The organic horizon was physically determined with common field methods and confirmed by an operational definition of greater than 40% loss-on-ignition (Soil Survey Staff, 2010). Mineral soil was excavated within a 0.25 m<sup>2</sup> frame, sieved to  $< 5$  cm. The soil mass for each mineral horizon was weighed to the nearest g using a MXX-10 portable electronic scale in the field (Denver Instruments, NY, USA). Two-kg bulk samples were collected from each horizon, air-dried to a constant weight, and sieved to correct the field measured soil horizon mass for water, rock and root masses. Horizon thicknesses were measured for all organic horizon and upper mineral soil samples. Rocks, twigs and roots  $> 1$  cm in diameter were removed from all organic horizon and mineral soil samples. Samples were air-dried to a

constant weight at 25 °C. Organic horizon samples were milled to < 2 mm and mineral soil samples were sieved to < 2 mm.

Earthworms were collected from 0.5 m × 0.5 m quantitative soil pits using the hand-sort method. Hand-sorting better estimates deep-burrowing species than alternative methods such as extraction with a hot mustard solution (Lawrence and Bowers, 2002). Earthworms were stored alive in their horizon material and identified live in the laboratory using a dichotomous key (Great Lakes Worm Watch, University of Minnesota, 2011). Classification of juveniles were made using identifiable physical features, supplemented with information of known adult species or raised until mature. Despite use of these measures, misidentification of juvenile earthworms of minor constituent species is possible, particularly from the genus *Aporrectodea*. To evacuate their digestive tract, earthworms were kept moist in empty containers for 24 hrs prior to weighing and cryodesiccation. Earthworms were weighed for dry weight (dw) biomass after lyophilization to a constant weight. Only earthworms with clitella (i.e., adults) were analyzed for TMM. The earthworms were grouped into four ecophysiological groups: Anecic (deep-burrowing, litter feeding), epi-endogeic (shallow-burrowing, soil and organic horizon-feeding), epigeic (shallow-burrowing organic horizon-feeding) and endogeic (shallow-burrowing soil feeding) earthworms (Bouché 1977; Lavelle 1979; Edwards and Bohlen 1996).

### 2.3 Physical and chemical soil properties

Soil pH was determined using a 2:5, soil : water slurry in 0.01 M CaCl<sub>2</sub>. The % SOM of each horizon was determined using loss on ignition, in which 4 g of soil was held at 475 °C for 8 hr. Data on soil properties are given in Supplemental Table 1. A modified Bouyoucos hydrometer method was used to quantify the particle size distribution (Gee and Bauder, 1986). In brief, the modified method involved using 30 g of soil treated with 30% w/w hydrogen peroxide to oxidize SOM and dispersed overnight with 100 mL of 0.08 M sodium hexametaphosphate. The soil slurry was brought to 1 L using deionized water in a graduated cylinder. Hydrometer measurements were taken at 30 s, 60 s, 1.5 hr, and 24 hr after 30 s of mixing. Clay contents were determined from the 1.5 hr and 24 hr measurements.

### 2.4 Acid-extractable TMM quantification and quality control

Soil and earthworm samples were digested following EPA method 3051A. For organic horizon and mineral soil analyses, 250 mg of air-dried material was used. Residual water mass in air-dried sub-samples was corrected for using oven-dried (105 °C for 3 d) samples. For earthworm analysis, an entire animal was lyophilized and typically weighed between 100 and 400 mg dry weight. These samples were digested 12 hr with 5 mL of reverse aqua regia (9:1, 70% HNO<sub>3</sub> : 30% HCl, Trace metal grade, Fisher Scientific) in 50 mL polypropylene centrifuge tubes at 25 °C. After 12 hr of degassing, the samples were microwave digested at 90 °C for 45 minutes in a CEM Mars microwave digestion system (CEM, Matthews, NC). The digestate was diluted 40 fold with micropore deionized water and analyzed with an Agilent 7700× ICP-MS (Agilent Technologies, Santa Clara, CA) for (Cu, Mo, Ni, Se, Zn, As, Cd, Hg, Pb, U). Every 25 samples included a digestion blank, a randomly spiked sample, a duplicate and a standard reference material (SRM). Digestion blanks were below detection limits of approximately 0.05 ng L<sup>-1</sup>. A random sample was

spiked with 50  $\mu\text{L}$  of multi-element standard 71A from Inorganic Ventures (Inorganic Ventures, Christiansburg, Virginia) and recoveries of the added elements ranged between 86 - 101% with an average of 93% for all spiked samples. Matching sample matrices from the National Institute of Standards and Technology were used: Montana Soil 2711 for mineral soil samples, Peach Leaves 1547 for organic horizon samples, and Mussel Tissue 2976 for earthworm tissue analysis (National Institute of Standards and Technology, Gaithersburg, MD). All metal concentrations for SRMs were within 9% of their certified values. Using the soil concentration and horizon mass, the pools of each metal in each horizon were calculated.

## 2.5 Statistical Analyses

Descriptive statistics for soil properties, TMM concentrations, and TMM pools were calculated in Matlab R2011b (MathWorks Inc, Natick, MA). Differences in soil properties at stands with and without earthworms were evaluated with the Kruskal-Wallis test and the Mann-Whitney U test. TMM concentrations, TMM pools, and bioaccumulation factors for earthworm tissues and soil samples were compared using two-sample t-tests when data were found to be normally distributed using the Lilliefors test (Lilliefors, 1967). For the figures and in text data, average values are given  $\pm 1$  standard error.

## 3 Results

### 3.1 Forest soil properties and TMM

Soils were primarily Inceptisols, (Cambisols) with shallow organic horizons and A, Ap, or A/E upper mineral soil horizons but Spodosols (Podzols) were also present. The average organic horizon depth was  $4 \pm 1$  cm in this study, while Juillerat et al. (2012) at similar elevations in the study region observed organic horizon depths of  $6 \pm 1$  cm. Soil pH was acidic, ranging from a low of 3.61 to a high of 5.09 (Supplemental Table 2). The average organic horizon pH ( $4.23 \pm 0.17$ ) was similar with the upper mineral soil ( $4.16 \pm 0.19$ ) ( $P = 0.41$ ). The % SOM ranged from a low of 7% in the mineral soil to a high of 80% in the organic horizon. The average % SOM was significantly greater for the organic horizon ( $60 \pm 4$  %) compared to the mineral soil ( $13 \pm 1$  %) ( $P < 0.01$ ). Soil texture was dominated by sand for all mineral soil horizons at all stands (Supplemental Table 2). The average particle size distribution was: sand =  $72 \pm 7$  %, silt =  $24 \pm 6$  % and clay =  $3 \pm 1$  %. Site 9 had an above-average proportion of sand ( $95 \pm 3$  %) ( $P < 0.05$ ) while Site 5 had a below-average sand proportion ( $50 \pm 5$  %) ( $P < 0.05$ ).

The acid-extractable soil TMM concentrations and pools are given in Supplemental Table 3 and Supplemental Table 4, respectively. Organic horizon TMM concentrations were similar to mineral soil TMM concentrations except for Cd and Zn, in which the organic horizon was higher than the mineral soil ( $P < 0.01$ ). Organic horizon TMM concentrations were similar among all 9 sites for Zn, Cd, and Pb but were significantly different for the remaining metals (Supplemental Table 3). Organic horizon TMM pools were significantly lower for sites 7 – 9 when compared to Sites 2 – 5 for all metals ( $P < 0.05$ ) with differences nearly two orders of magnitude for As, Se, Mo, Hg, Pb and U (Supplemental Table 3 and 4). Mineral soil TMM concentrations and pools varied for all metals but were less than one order of magnitude in

difference (Supplemental Table 3 and 4). Organic horizon and mineral soil TMM concentration was poorly correlated with soil pH. The combined organic horizon and mineral soil % SOM data set was significantly correlated with concentrations of As ( $r = -0.54$ ), Cd ( $r = 0.72$ ), Hg ( $r = 0.57$ ), and Zn ( $r = 0.56$ ), ( $P < 0.05$  for each, Supplemental Table 5).

### 3.2 Earthworm species presence and properties

Earthworms were present at all 9 sites, in 30 of the 45 stands examined in this study. The earthworm species collected at each stand are given in Supplemental Table 1 and the total number of adult and juvenile earthworms is given in Supplemental Table 6. In total, seven species were identified in varying assemblages, covering all four ecophysiological groups (Bouche, 1977; Edwards and Bohlen, 1996). The only epigeic earthworm collected was *Dendrobaena octaedra*. Endogeic species collected were *Aporrectodea rosea*, *Aporrectodea tuberculata*, and *Octolasion cyaneum*. Epi-endogeic species were *Amyntas agrestis* and *Lumbricus rubellus*. The only observed anecic earthworm was *Lumbricus terrestris*.

Although considered endogeic, *Aporrectodea rosea* was commonly found in the Oe and Oa horizons of the organic horizon. *Lumbricus terrestris* were found at depths  $> 30$  cm and commonly in permanent burrows in Bw horizons. *A. rosea* had the greatest population densities while *L. terrestris* and *Octolasion cyaneum* had the lowest (Figure 2a). The individual earthworms dw biomasses of *Amyntas agrestis* and *L. terrestris* were significantly greater than the other species (Figure 2b). Due to the high population density and high average dw biomass, *A. agrestis* was found to have significantly greater dw biomass per m<sup>2</sup>, almost 3 times greater than most other species (Figure 2c). The organic horizon pH ( $4.68 \pm 0.14$ ) and mineral soil pH ( $4.76 \pm 0.08$ ) at epi-endogeic inhabited stands were significantly greater than the average organic horizon pH ( $3.86 \pm 0.18$ ) and average mineral soil pH ( $3.76 \pm 0.21$ ) at stands with all other ecophysiological groups ( $P < 0.05$ ). Earthworms from the *Amyntas* genus are considered invasive species due to the fact that they out-compete indigenous soil fauna for resources as well as their negative impacts on forest leaf-litter decomposition and nutrient cycling (Zhang et al., 2010; Snyder et al., 2011; Greiner et al., 2012).

Soils inhabited by *A. agrestis* had significantly thinner organic horizon ( $1.7 \pm 0.5$  cm) compared to the average depth at stands with other earthworms ( $5.9 \pm 0.9$  cm) ( $P < 0.01$ ). Organic horizon TMM concentrations at stands with and without earthworms varied significantly, but did not exhibit a general trend. Organic horizon Cu and Ni concentrations were greater at stands with earthworms while organic horizon Se, Hg and Pb concentrations were lower at stands with earthworms (Supplement Table 7,  $P < 0.05$ ). Organic horizon pools of Cu, Mo, Se, Cd, Hg and Pb were lower at stands with earthworms compared to stands without earthworms ( $P < 0.05$ , Supplemental Table 8). Mineral soil Cu, Zn, Cd and U concentrations and pools were greater at stands with earthworms compared to stands without earthworms ( $P < 0.05$ , Supplemental Table 8).



### 3.4 Earthworm TMM concentrations and pools

The strong acid-extractable earthworm tissue TMM concentrations for each species are given in Table 2. *Octolasion cyaneum* had greater tissue concentrations of Cu, Ni, As and U than the average earthworm tissue concentration (Table 2,  $P < 0.01$ ). *Lumbricus terrestris* had lower tissue concentrations of Mo, Ni, Se, As, Cd, Hg, Pb, and U than the overall average tissue concentration (Table 2,  $P < 0.05$ ). Despite being from the same genus, *Aporrectodea rosea* and *Aporrectodea tuberculata* had significantly different Cu, Se, Cd, Hg, Pb and U tissue concentrations, commonly varying by as much as 50% ( $P < 0.05$ , Table 2). Similarly, *L. rubellus* and *L. terrestris* tissue concentration of Ni, Se, As, Cd, Hg, Pb and U varied significantly ( $P < 0.05$ , Table 2). Endogeic and epigeic species generally had higher tissue concentrations of As, Cd, Hg, Pb and U than epi-endogeic and anecic earthworms (Table 2). Despite having higher than average tissue concentrations, *O. cyaneum* had lower tissue pools of Cu, Mo, Ni, Se, Zn, Cd and Pb than the average tissue pool (Table 3,  $P < 0.05$ ). *Amyntas agrestis* had greater tissue pools of Cu, Mo, Zn, and As than the overall average earthworm tissue pool (Table 3,  $P < 0.05$ ). Earthworm tissue TMM concentrations were poorly correlated with their respective soil's TMM concentration and pH of their respective soil horizon, with  $R^2$  values  $< 0.15$  for all regressions (Supplemental Table 5). Earthworm tissue Ni, Se, As, and U concentrations were negatively correlated with their respective soil's % SOM ( $R^2 > 0.25$ ,  $P < 0.05$ , Supplemental Table 5).

The variation in earthworm TMM tissue concentrations and pools was investigated for the 9 sites for each of the earthworm species. Generally, Cu, Ni, Mo, Zn, As, Cd, and U tissue TMM concentrations did not vary consistently among sites across species (Supplemental Figure 1 and 2). Earthworm tissue Se, Hg and particularly Pb concentrations were significantly different among sites for most species (Supplemental Figures 1 and 2). Because of the varying abundance of earthworms at each site, the tissue TMM pools could only be compared for *A. agrestis* and *A. rosea* (Supplemental Figure 3). *A. agrestis* at Site 8 had higher tissues pools of Cu, Mo, Ni, Se, Zn, As, Cd, and U than at Site 9. Similarly, *A. rosea* at Site 4 had higher tissues pools of Cu, Mo, Ni, Se, Zn, As, Cd, Hg and U than Sites 1, 2, 3, and 6 (Supplemental Figure 3).

The bioaccumulation factor (BAF), or synonymous bioconcentration factor (Ernst et al. 2008) and Biota-to-Soil Accumulation Factor (Dai et al. 2004), is a useful metric to compare the concentration of TMM in earthworm tissue relative to the soil material they consume (Burton et al. 2006). The BAF values are displayed by species in Figure 3. All earthworms had BAF values within one order of magnitude of each other for all metals. BAFs exhibited low variance across most metals (CV 9 – 18 %), except for Se (CV 544 %), Cd (CV 654 %), Hg (CV 256 %) and Pb (59 %) (Figure 3). When considering the BAFs by ecophysiological groups, endogeic and epigeic generally had higher BAFs while the anecic earthworm *Lumbricus terrestris* had the lowest BAFs. This is particularly evident for Se, Cd and Hg BAFs (Figure 3).

## 4 Discussion

### 4.1 Forest soil TMM

Acid-extractable concentrations of As, Cu, Zn, Mo, Se, and U were within the typical range of soils in the United States (Adriano 2001; Alloway 2013, Friedland et al. 1986, Evans et al. 2005, Kaste et al. 2006, Jullierat et al. 2012, Richardson et al. 2013, Richardson et al. 2014). Organic horizon and mineral soil TMM concentrations generally reflected their relative abundance in granitic and meta-sedimentary rocks that compose the soils of northern New England (Jersak et al. 1997; Adriano 2001; Landre et al. 2010). Sites were located away from point sources of pollution and TMM concentrations likely varied among sites due to factors not investigated in this study such as soil development and land-use history. However, organic horizon concentrations of Cd and Pb were greater than typical values for United States soils due to increased atmospheric deposition from regional sources (Siccama and Smith, 1978; Friedland et al., 1986; Driscoll et al. 2007; Richardson et al., 2015).

Soil pH was poorly correlated with organic horizon and mineral soil TMM concentrations ( $P > 0.10$ ). The range in soil pH (3.87 to 5.20) may not have been large enough to evaluate the role of soil pH on soil TMM accumulation. However, % SOM was linearly correlated with Cd, Hg and Zn (Supplemental Table 5,  $P < 0.05$ ). The positive correlation between % SOM and Group 12 elements (Cd, Hg and Zn) is due to complexation (Adriano 2001), likely caused by specific functional groups such as carboxylates, sulfides and thiols (e.g. Skyllberg et al. 2003; Obrist et al. 2011). Arsenic may have been negatively correlated with % SOM due to greater leaching to the mineral soil resulting from anion exclusion. Moreover, the organic horizon had lower As concentrations compared to the mineral soil because As is primarily derived from weathering minerals rather than atmospheric deposition (Adriano 2001).

Organic horizon and mineral soil TMM concentrations and pools varied among sites and with earthworm presence. Organic horizon and mineral soil TMM concentrations generally did not follow any correlation with pH or %SOM, suggesting other site-specific processes are most influential. Other potentially influential site-specific factors include parent material, land-use history, and vegetation. Broadly, stands with more developed soils and higher % deciduous vegetation had higher TMM pools. Considering differences between stands with and without earthworms, the TMM concentrations and pools were greater in the mineral soil but lower in the forest floor at stands with earthworms, suggesting earthworms have redistributed TMM (Supplemental Table 7 and 8). This may have occurred from bioturbation of soil horizon or increased organic horizon decomposition. However, it is difficult to eliminate other co-varying factors such as soil properties, site characteristics, earthworm assemblages, limited sample size and other unknown factors such as temporal variability. The effect of earthworms on soil morphological and metal accumulation in soil is discussed at length in a separate study on a subset of the sites that have known land-use histories.



## 4.2 Earthworm species

The cause of the abundance and varying assemblages of earthworms across the 9 sites appears related to land-use. Stands with earthworms were generally near human developments and likely introduced by fishing bait release, import of organic or soil material, surrounding agriculture, timber harvesting, or domestic gardening (Edwards and Bohlen, 1996; Bohlen et al., 2004; Frelich et al., 2006; Görres and Melnichuk, 2012). All species in this study are considered to be exotic and have been found in eastern Canada, the great lakes states and the northern mid-Atlantic states (Hale et al., 2005; Addison et al., 2009; Snyder et al., 2011). Hale et al. (2005) observed nearly the same species composition as observed at our stands in northern Minnesota, > 2000 km away, suggesting these exotic species have been widely distributed by humans. The agricultural legacy of New England may also have contributed to the dispersion and present range of these earthworms. *Lumbricus rubellus* and *Lumbricus terrestris* were expected to be most widespread due to agricultural legacy and fishing but they were not as abundant as *Aporrectodea rosea*. The wide distribution of *A. rosea* is noteworthy, particularly in the Spodosols at site 5, which were excavated at least 500 m away from the nearest road or human development. However, nearby forests had been previously harvested for lumber, suggesting the importance of logging in the movement of *Aporrectodea* species. A study by Görres and Melnichuk, (2012) is the first to report invasive populations of *Amyntas spp.* in forests of Vermont. These were later identified as *A. agrestis* (Görres, *personal communication*, 2013; Görres et al., 2014). The organic horizon was significantly thinner at stands inhabited by *A. agrestis* ( $1.7 \pm 0.5$  cm) compared to all other earthworm-inhabited stands ( $5.9 \pm 0.9$  cm). Although this may have arisen from random sampling error, many earthworms, have been known to consume the entire organic horizon, particularly those from the Megascolidae family, (Edwards and Bohlen, 1996; Bohlen et al., 2004; Snyder et al., 2011).

## 4.3 Earthworm TMM concentrations and pools

Our results demonstrate that earthworms in non-urban, unpolluted sites can have considerably high TMM concentrations. The average earthworm tissue Cd, Cu, Ni, Pb and U concentrations in our study were greater than concentrations observed for *Nicodrilus caliginosus* in peri-urban and urban areas of Siena, Italy (Nannoni et al., 2014). Moreover, earthworm tissue Cd and Pb concentrations at our remote and non-urban sites matched or exceeded those found in human contaminated and amended soils (Suthar et al., 2008; Latif et al., 2013). When earthworm tissue concentrations were considered by their ecophysiological group, endogeic and epigeic species generally had higher tissue concentrations of toxic TMM (As, Cd, Hg, Pb and U) than epi-endogeic and anecic earthworms. This follows several other studies in which epi-endogeic, and endogeic earthworms had higher tissue TMM accumulation than epigeic or anecic earthworms (Ireland, 1979; Ernst et al., 2008; Suthar et al., 2008; Latif et al., 2013). Morgan and Morgan (1999) observed the endogeic *Aporrectodea caliginosa* had up to 4 times higher tissue concentrations of Cd, Cu, and Pb than the epigeic *Lumbricus rubellus*. The higher TMM tissue concentrations in epigeic and endogeic than anecic earthworms may be from site-specific factors (Suthar et al., 2008; Zhang et al., 2009) or preferential consumption of decomposed SOM containing higher TMM concentrations (Ireland, 1979; Latif et al., 2008).

Earthworms are preferential feeders (Edwards and Bohlen, 1996) and focused consumption of the Oa and A horizon by endogeic and epi-endogeic earthworms may have caused higher tissue TMM concentrations than anecic and epigeic earthworms, which prefer fresh litter, Oi horizons, and Oe horizons (Morgan and Morgan, 1999; Hobbelen et al., 2006; Suthar et al., 2008; Latif et al., 2013). Earthworm tissue concentrations were poorly correlated with their respective soil horizon concentration (Supplemental Table 5), which matches findings by Beyer and Cromartie (1987) and Maleri et al., (2008). However, the significant correlation between earthworm tissue concentrations of Ni, Se, As, and U and % SOM of their respective horizon suggests these metals are derived from consumption of the mineral soil, which is low in SOM. An alternative hypothesis is soil acidity controls bioavailability and uptake of TMM (Burton et al., 2006; Lukkari et al., 2006; Suthar et al., 2008; Alloway, 2013) but our study did not find a correlation with soil pH and TMM concentrations in earthworms. The effect of other site-specific factors (e.g. vegetation, microtopography) and individual-specific factors (e.g. earthworm age) were not assessed and may have influenced earthworm TMM accumulation.

Tissue concentrations of Cu, Mo, Ni, Zn, As, Cd, and U did not exceed concentrations known to be harmful to vertebrates, which follows previous studies in other regions (e.g. Beyer and Cromartie, 1987; Ernst et al. 2008; Zhang et al., 2009). However, the average earthworm tissue concentrations of Hg, Se and Pb exceeded the Maximum Tolerable Levels in feed for mice and poultry as described by the Committee on Minerals and Toxic Substances in Diets and Water for Animals from the National Research Council (Klasing et al., 2005). Moreover, the average earthworm Se tissue concentrations exceeded the US EPA's Ecological Soil Screening Level for Se (soil invertebrates  $4 \text{ mg kg}^{-1}$ ) but no concentration has been set for Hg (US EPA, 2007). This suggests that earthworms could be an unrecognized source of potentially hazardous concentrations of Hg, Se and Pb to predators such as small mammals, ground-foraging avians and potentially higher trophic levels (Talmage and Walton, 1993; Loss et al. 2012).

Recent studies describing the terrestrial trophic transfer of mercury in northern New England have not considered earthworms as a potential source (Rimmer et al. 2010; Townsend et al. 2014). Earthworm Hg tissue concentrations in our study ( $0.19 - 0.88 \text{ mg kg}^{-1}$ ) exceeded tissue Hg concentrations in nearly all other terrestrial invertebrates ( $0.01 - 0.20 \text{ mg kg}^{-1}$ ) reported in Rimmer et al. (2010). For example, opportunistic foragers in northern New England, such as red-backed salamanders and Bicknell's Thrush, have been found with inexplicably high Hg tissue and blood concentrations, respectively (Rimmer et al., 2010; Townsend et al., 2014). As a generalist predator, red-back salamanders have been observed to consume large numbers of exotic earthworms (Maerz et al., 2005) and this may be an important Hg source to them and other ground-foraging mammals and birds as well (Talmage and Walton, 1993).

By only examining TMM tissue concentrations, the pools of TMM in earthworms have been overlooked despite representing a significant ecosystem pool and potentially flux of TMM in forests of the northeastern US. When present, epigeic + epi-endogeic tissue pools exceeded the organic horizon pools for Se, Zn, Cd, and Hg (Figure 4). In particular, earthworm tissue Cd pool was 12 times the pool in the organic horizon. The comparable

TMM pools per unit area in the earthworms and organic horizons may have arisen as a result of: 1) high dw biomass per m<sup>2</sup>, particularly for *Amyntas agrestis*, which has a high bioaccumulation of Se, Zn, Cd and Hg, or 2) earthworms have assimilated TMM from both the organic horizon and the mineral soil. In either case, these findings highlight the importance of earthworms in soil biogeochemistry and suggest that when present, earthworms should be considered for TMM cycling in forests. An additional implication is their potential effect on the elevated pools of Cd, Pb and Hg from decades of anthropogenic emissions may no longer be held in the organic horizon and mineral soil but may be incorporated into the terrestrial biomass. Lastly, it demonstrates that earthworms may contribute to the spatial variability of soil TMM concentrations at stands with earthworms by concentrating metals into small areas upon their expiration. This may be potential source of error in soil sampling when soil cores are < 10 cm in diameter. Using a 15 × 15 cm organic horizon template and 50 cm × 50 cm soil pit likely has overcome this potential of under-sampling by examining more soil area.

#### 4.4 Earthworm TMM bioaccumulation

From Fig. 3, the overall average BAF values for the TMM were Cd > Se > Hg > Zn > Pb > U > 1.0 > Cu > As > Mo > Ni. Following previous studies (e.g. Ireland, 1979; Burton et al., 2006 and Ernst et al., 2008), BAF values of 1 show that soil concentrations exceed earthworm TMM tissue concentrations, suggesting dilution or non-accumulation and BAF values > 1 suggest active bioaccumulation by earthworms. Using this premise, Cu, Mo, Ni, As, Pb and U were not bioaccumulated in earthworm tissues while Cd, Se, Hg, and Zn were strongly bioaccumulated. The order of bioaccumulation was similar to the findings of Ireland et al., (1979), Dai et al., (2004), Ernst et al., (2008), and Latiff et al., (2013) despite different assemblages of species being analyzed. Moreover, Cd, Hg, and Pb, BAF values for *Aporrectodea rosea*, *Lumbricus rubellus*, *Lumbricus terrestris* and *Octolasion cyaneum* in this study were similar with values from Ernst et al. (2008). These results support the finding that ecophysiological groups bioaccumulate TMM differently, but still have a similar bioaccumulation order of Cd > Hg > Pb in uncontaminated soils.

One possible mechanism controlling BAF values is biomass dilution, in which larger earthworms have more biomass to dilute TMM tissue concentrations (Zhang et al., 2009). However, physiological regulation of uptake and assimilation of TMM as a controlling mechanism is the consensus of previous studies (Ireland, 1975; Morgan and Morgan, 1990; Burton et al., 2006). Earthworms may actively decrease TMM from assimilation into tissue with specialized organs such as calciferous glands in the Lumbricidae family may aid in excretion of TMM within calcite granules (Morgan and Morgan, 1990; Edwards and Bohlen, 1996; Dai et al., 2004; Burton et al., 2006; Udovic and Lestan, 2007; Sizmur and Hodson, 2009). The inclusion of TMM into calcite granules may be most important for metals such as Cu, Zn and Pb (Morgan and Morgan, 1999; Brinza et al., 2014) and for certain genera such as *Lumbricus* (Morgan and Morgan, 1990). Moreover, certain tissues can promote TMM accumulation; Morgan and Morgan (1990) found the posterior alimentary canal of earthworms, particularly of the genus *Aporrectodea*, had accumulated significantly higher concentrations of Cd, Pb and Zn compared to the other tissues and attributed it to the chloragogenous tissues that line their intestine (Ireland 1975; Spurgeon

and Hopkin, 1999; Dai et al., 2004; Giovanetti et al., 2010). It can be surmised that digestive tracts that are longer or have more surface area may lead to greater accumulation in earthworms. Surface area for uptake in earthworm tissues may be greater in earthworms with more folding of the typhlosole, which has been observed to vary among species and ecophysiological groups (Wu 1939; Thakuria et al., 2010). In addition, earthworm age may also affect TMM accumulation as younger earthworms have been observed to have higher assimilation rates of ingested toxic metals and maintain higher tissue concentrations (Bengtsson and Rundgren, 1992; Neuhauser et al., 1995; Lukkari et al., 2006; Zhang et al., 2009). Lastly, the speciation and sorption of the TMM may control their uptake. For example, the TMM that generally exist in soils as oxyanions, specifically, As, Mo, and U, were not strongly bioaccumulated by earthworms, this has been observed in previous studies (Langdon et al., 2003; Giovanetti et al., 2010). Moreover, Cu and Ni may have been only as an inorganic precipitant as opposed to a dissolved or a complexed form, which are more bioavailable for uptake (Adriano, 2001; Hobbelen et al., 2006; Lukkari et al., 2006; Maleri et al., 2008; Alloway, 2013). Similarly, Nannoni et al., (2014) did not find Pb to be bioaccumulated by earthworms in urban and peri-urban areas likely due to strong sorption to inorganic components such as silicate and Fe-oxyhydroxides. However, the strong acid-digestion method employed for soil concentrations in this study did not provide any information on their bioavailability to earthworms.

## 5. Conclusions

This study assessed the TMM concentrations and pools, mass per unit area, of soils and earthworm tissues from northern New England. Our results demonstrate that earthworms may attain tissue concentrations of Hg, Se and Pb that are potentially hazardous to small mammals and ground-foraging birds, without point source contamination. There were observable differences in TMM accumulation among ecophysiological groups: endogeic and epigeic generally had higher tissue concentrations of toxic metals than epi-endogeic and anecic earthworms. We attributed this to preferential consumption of SOM with varying concentrations of TMM. From the bioaccumulation factors, it appears that earthworms strongly bioaccumulated Se, Zn, Cd, and Hg. In addition to the findings on varying concentrations, our study is one of the first to show that earthworms can be a considerable ecosystem pool of TMM. When epigeic + epi-endogeic tissue TMM pools were compared with organic horizon pools, earthworm pools were comparable or greater. This was most evident for Hg, Se and Cd and may represent a significant unaccounted flux within forested ecosystems. Our BAF and TMM pool results from sites inhabited by the invasive *Amyntas agrestis* suggest it had the most considerable impact on TMM cycling in forest soils of the earthworms in this study. Further research is necessary to determine the fate of TMM accumulated in the tissues of exotic earthworms; they may be either returned to the soil in a more mobile phase, may bioaccumulate in earthworm predators or be retained in stable SOM.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

We are grateful for the technical and laboratory assistance provided by Paul Zeitz and Janet Towse. This research was funded by a Porter Fund award to Andrew Friedland. Brian Jackson and the Dartmouth Trace Element Analysis Laboratory are partially supported by NIH grant P42 ES007373.

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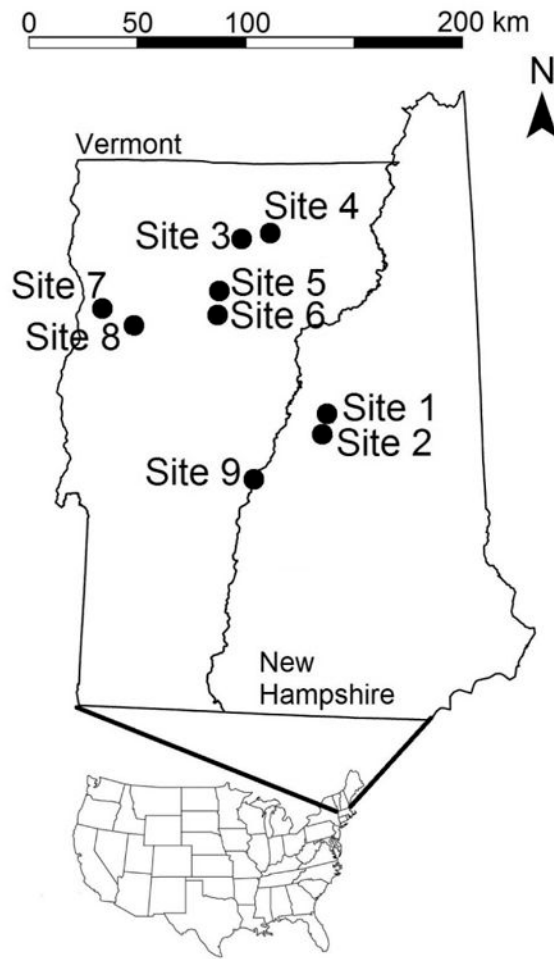
**Highlights**

The first survey of trace elements in exotic earthworms in New England, USA

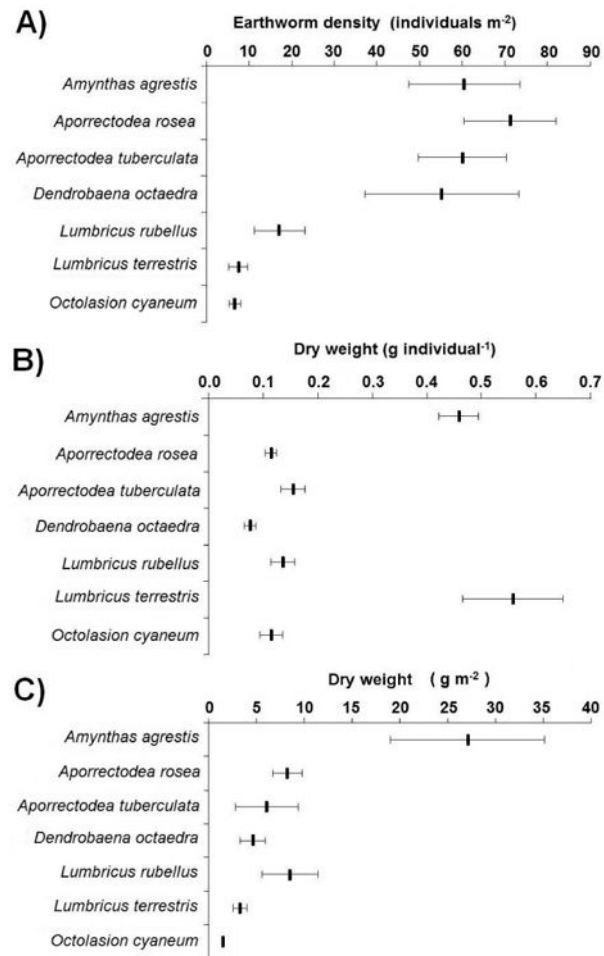
Earthworms in uncontaminated soils strongly bioaccumulated Se, Zn, Cd, and Hg.

Concentrations of Pb, Hg, and Se in earthworms may be hazardous to predators.

Earthworm tissue Zn, Cd and Hg pools exceeded organic horizon pools.

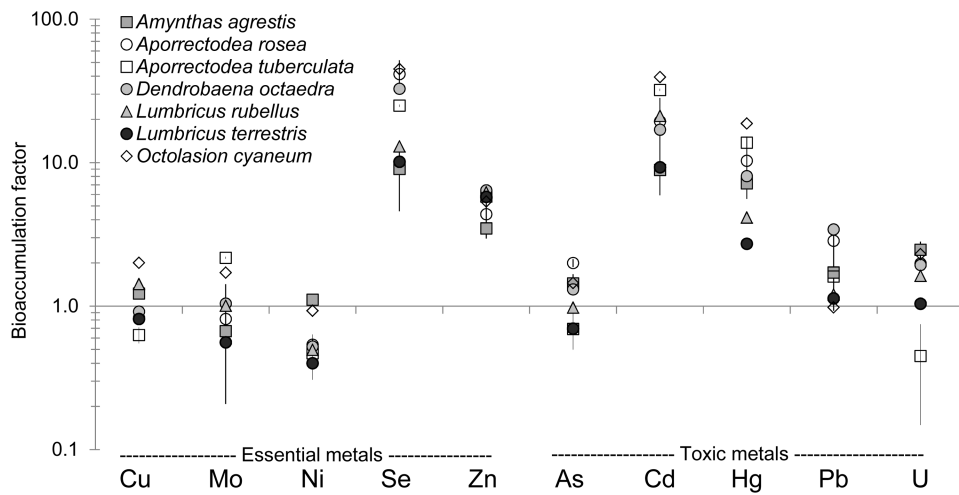


**Figure 1.**  
Sampling site locations.



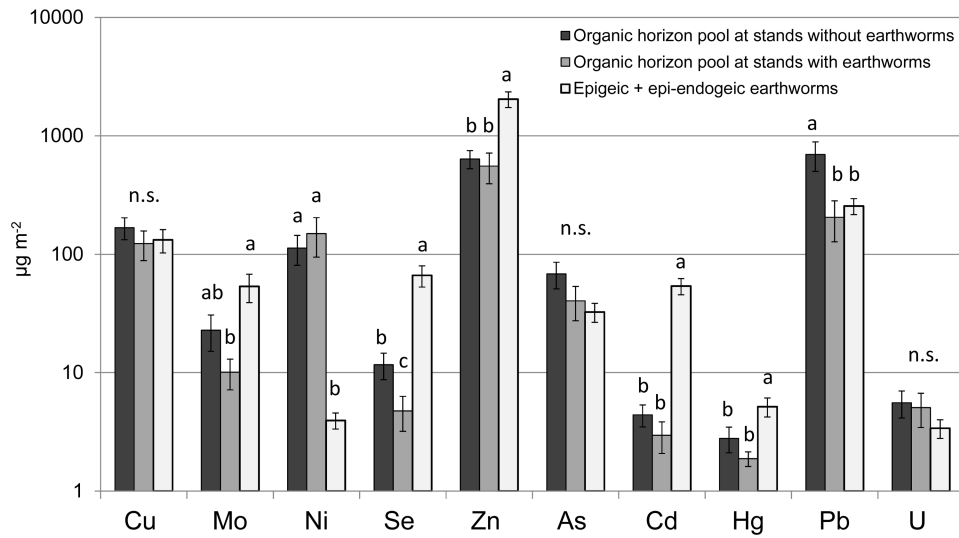
**Figure 2.** (A) Earthworm density per square meter at stands when observed. (B) Earthworm dry weight per individual. (C) Earthworm dry weight biomass per m<sup>2</sup> when observed.





**Figure 3.**

A logarithmic comparison of mean bioaccumulation factors for each earthworm species  $\pm 1$  S.E. Bioaccumulation factors were calculated as earthworm concentration ( $\text{mg kg}^{-1}$ ) divided by soil concentration ( $\text{mg kg}^{-1}$ ). Epigeic and epi-endogeic earthworms have gray symbols, endogeic earthworms have white symbols and anecic earthworms have black symbols.



**Figure 4.**

A logarithmic comparison of mean organic horizon TMMs pools (mass per unit area) at stands with and without earthworms to epigeic+epi-endogeic TMM tissue pools when observed. Average values are given in  $\mu\text{g m}^{-2} \pm 1 \text{ S.E.}$  The non-parametric Kruskal-Wallis test was used to compare the three pools for each TMM. Letters (a, b, c) are used to group the three pools for each metal ( $P < 0.05$ ) and (n.s.) denotes no significant difference.

Table 1

## Site Description

Site #	Site	Latitude	Longitude	Elevation (m)	Soil series present <sup>†</sup>	Earthworm species present <sup>‡</sup>
1	Lake Armington	43° 57' 22"	71° 57' 43"	427	Becket-Acton	B, F
2	Upper Baker Pond	43° 54' 21"	71° 59' 39"	284	Tunb ridge-Acton	B, D
3	Great Hosmer Pond	44° 41' 34"	72° 22' 13"	367	Ver shire-Buckland	B, D, E
4	Lake Parker	44° 43' 13"	72° 14' 33"	411	Cabot-Ver shire	B, E
5	Hardwood Pond	44° 28' 14"	72° 30' 14"	488	Tunbrid ge-Vershire	B
6	Curtis Pond	44° 23' 35"	72° 29' 32"	381	Hartland- Glover	B, C, D
7	UVM Hort-Farm	44° 25' 53"	73° 12' 1"	73	Duane-Deerfield	A, C, D, E, F, G
8	Huntington Audobon	44° 20' 49"	72° 59' 49"	199	Ad ams-Himesburg	A, B, C, G
9	Montshire Museum	43° 41' 52"	72° 18' 25"	155	Hart land- Hitchcock	A, B, D, F, G

<sup>†</sup> Soil series from mapped units from [websoilsurvey.gov](http://websoilsurvey.gov) accessed 14<sup>th</sup> Nov. 2013.

<sup>‡</sup> A. *Amyntas agrestis*, B. *Aporrectodea rosea*, C. *Aporrectodea tuberculata*, D. *Dendrobaena octaedra*, E. *Lumbricus rubellus*, F. *Lumbricus terrestris*, G. *Octolasion cyaneum*.

Table 2

Mean acid-extractable tissue concentrations of essential and toxic trace metals for each species. Mean values are given  $\pm$  1 S.E. in mg kg<sup>-1</sup>.

Earthworm	†	n‡	Essential					Toxic					
			Cu	Mo	Ni	Se	Zn	As	Cd	Hg	Pb	U	
			mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>
<i>Amyntas agrestis</i>	EE	20	19 ± 2	0.35 ± 0.02	7.4 ± 0.5	2 ± 1	152 ± 49	2.3 ± 0.2	3.1 ± 1.3	0.43 ± 0.14	21 ± 11	0.24 ± 0.04	
<i>Aporrectodea rosea</i>	En	33	10 ± 1	0.51 ± 0.02	3.8 ± 0.9	14 ± 1	270 ± 49	4.1 ± 0.2	8.9 ± 0.2	0.88 ± 0.09	43 ± 5	0.47 ± 0.02	
<i>Aporrectodea tuberculata</i>	En	7	11 ± 2	0.54 ± 0.15	4.8 ± 0.4	6 ± 2	222 ± 22	3.5 ± 0.3	4.6 ± 1.0	0.41 ± 0.10	30 ± 7	0.24 ± 0.06	
<i>Dendrobaena octaedra</i>	Ep	12	12 ± 2	0.57 ± 0.07	3.6 ± 0.6	7 ± 3	333 ± 41	2.5 ± 0.7	5.9 ± 1.2	0.52 ± 0.17	43 ± 20	0.25 ± 0.05	
<i>Lumbricus rubellus</i>	EE	9	14 ± 1	0.45 ± 0.03	6.6 ± 0.8	6 ± 1	247 ± 24	4.6 ± 0.4	4.5 ± 0.6	0.29 ± 0.03	24 ± 5	0.60 ± 0.08	
<i>Lumbricus terrestris</i>	An	8	12 ± 1	0.30 ± 0.03	2.5 ± 0.3	2 ± 1	268 ± 39	0.8 ± 0.1	2.7 ± 0.4	0.19 ± 0.03	14 ± 4	0.09 ± 0.01	
<i>Octolasion cyaneum</i>	En	5	35 ± 3	0.53 ± 0.01	13.0 ± 0.7	10 ± 2	194 ± 14	5.8 ± 0.2	5.0 ± 1.2	0.54 ± 0.24	20 ± 8	0.81 ± 0.05	
Overall average		94	15 ± 4	0.47 ± 0.05	5.7 ± 1.6	7 ± 2	245 ± 28	3.4 ± 0.8	5.5 ± 1.4	0.52 ± 0.13	29 ± 6	0.40 ± 0.12	
Kruskal-Wallis P-value			**	*	**	**	*	**	**	*	*	**	

† Earthworm ecophysiological group (Ep = epigeic, En = endogeic, EE = epi-endogeic, An = anecic)

‡ n is the number of adult earthworms analyzed.

\* indicates P < 0.05,

\*\* indicates P < 0.01

Mean acid-extractable earthworm tissue pools, mass per unit area, of essential and toxic TMM for each species. Mean values  $\pm$  1 S.E. are given in  $\mu\text{g m}^{-2}$ .

Table 3

Earthworm	$\dagger$	$n^{\ddagger}$	Essential					Toxic				
			Cu	Mo	Ni	Se	Zn	As	Cd	Hg	Pb	U
			$\mu\text{g m}^{-2}$	$\mu\text{g m}^{-2}$	$\mu\text{g m}^{-2}$	$\mu\text{g m}^{-2}$	$\text{mg m}^{-2}$	$\mu\text{g m}^{-2}$	$\mu\text{g m}^{-2}$	$\mu\text{g m}^{-2}$	$\mu\text{g m}^{-2}$	$\mu\text{g m}^{-2}$
<i>Amyntas agrestis</i>	EE	8	488 $\pm$ 42	9.3 $\pm$ 2.7	215 $\pm$ 75	37 $\pm$ 10	3.8 $\pm$ 0.7	74 $\pm$ 17	95 $\pm$ 32	8.2 $\pm$ 2.1	460 $\pm$ 98	6.5 $\pm$ 1.9
<i>Aporrectodea rosea</i>	En	21	82 $\pm$ 14	4.1 $\pm$ 0.7	28 $\pm$ 4.9	122 $\pm$ 29	2.3 $\pm$ 0.4	36 $\pm$ 8	74 $\pm$ 14	8.0 $\pm$ 2.0	326 $\pm$ 59	4.0 $\pm$ 1.1
<i>Aporrectodea tuberculata</i>	En	3	61 $\pm$ 21	3.0 $\pm$ 1.6	18 $\pm$ 9	67 $\pm$ 28	1.9 $\pm$ 0.5	26 $\pm$ 15	55 $\pm$ 40	4.6 $\pm$ 3.3	315 $\pm$ 89	1.5 $\pm$ 0.9
<i>Dendrobaena octaedra</i>	Ep	5	38 $\pm$ 15	1.7 $\pm$ 0.6	10 $\pm$ 4	21 $\pm$ 8	1.2 $\pm$ 0.3	7 $\pm$ 3	21 $\pm$ 6	1.5 $\pm$ 0.6	125 $\pm$ 58	0.6 $\pm$ 0.2
<i>Lumbricus rubellus</i>	EE	7	116 $\pm$ 39	3.9 $\pm$ 1.3	56 $\pm$ 19	51 $\pm$ 14	2.0 $\pm$ 0.4	39 $\pm$ 7	38 $\pm$ 13	2.5 $\pm$ 0.9	197 $\pm$ 97	5.1 $\pm$ 1.7
<i>Lumbricus terrestris</i>	An	7	38 $\pm$ 9	1.0 $\pm$ 0.2	10 $\pm$ 3	7 $\pm$ 1	0.8 $\pm$ 0.1	3 $\pm$ 1	8 $\pm$ 1	0.6 $\pm$ 0.1	42 $\pm$ 9	0.3 $\pm$ 0.1
<i>Octolasion cyaneum</i>	En	3	17 $\pm$ 2	0.3 $\pm$ 0.1	6 $\pm$ 1	4 $\pm$ 1	0.2 $\pm$ 0.1	3 $\pm$ 1	2 $\pm$ 1	0.5 $\pm$ 0.2	8 $\pm$ 3	0.3 $\pm$ 0.1
Overall average			132 $\pm$ 29	4.1 $\pm$ 0.6	54 $\pm$ 14	66 $\pm$ 14	2.0 $\pm$ 0.3	33 $\pm$ 6	54 $\pm$ 8	5.2 $\pm$ 0.9	255 $\pm$ 39	3.4 $\pm$ 0.6
Kruskal-Wallis P-value			**	**	**	**	**	**	**	**	**	**

$\dagger$  Earthworm ecophysiological group (Ep = epigeic, En = endogeic, EE = epi-endogeic, An = anecic).

$\ddagger$  n is the number of sites.

\* indicates  $P < 0.05$  and

\*\* indicates  $P < 0.01$