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Growth and reproduction of the earthworm *Eisenia fetida* after exposure to leachate from wood preservatives

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Abstract

Wood preserved with chromated copper arsenate (CCA) and alkaline copper quaternary (ACQ) was mixed with artificial rainwater, to generate leachates containing As, Cr and Cu. Then, leachates were applied to two soils at rates of 13–169 mg As kg^{-1} soil (dry weight basis), 12–151 mg Cr kg^{-1} and 10–216 mg Cu kg^{-1}. Metal bioavailability was evaluated after 28 days using the earthworm *Eisenia fetida* (Savigny). Metal concentrations in earthworm tissue ranged from negligible to 80 mg As kg^{-1} (dry weight basis), 89 mg Cr kg^{-1} and 90 mg Cu kg^{-1}, which appeared to be non-lethal to *E. fetida*. There was less Cu available to earthworms in the Courval soil (pH 7.8) than the Châteauguay soil (pH 6.8), but earthworm growth and reproduction were not affected by exposure to Cu from ACQ-treated wood. In contrast, earthworms exposed to As, Cr and Cu from the CCA-treated wood gained weight more quickly in the Courval soil (1.3–21 mg g^{-1} initial biomass days) than in the Châteauguay soil (0.2–7.8 mg g^{-1} day^{-1}), but fewer than 20% of the cocoons deposited by the faster-growing earthworms hatched by the end of the 56 days ecotoxicology test. It appeared that *E. fetida* can allocate more energy to growth than reproduction, delaying cocoon development and hatching in some situations. Further information is needed on the soil factors that may induce such behavior, as it can affect the interpretation of results from the earthworm ecotoxicology test.

Keywords: Soil pH; Wood preservative; Metal bioavailability; *Eisenia fetida*; Growth; Reproduction; Arsenic; Copper; Chromium

1. Introduction

Chromated copper arsenate (CCA) is an inorganic waterborne wood preservative that was widely used in North America for approximately 60 years to protect wooden structures from insect damage and decay. Wood treated with CCA was used for many outdoor residential and commercial constructions, such as decks, telephone and electric poles, docks, picnic tables, playground structures, railway tracks and landscape timbers (Stilwell et al., 2003). Concerns about human exposure to arsenic, a known carcinogen, led the wood preserving industry to voluntarily withdraw CCA-treated wood from the residential construction market in the United States and Canada in 2003 (USEPA, 2002; Health Canada, 2003). Other inorganic wood preservatives, such as chromated copper boron, alkaline copper quaternary (ACQ), ammoniacal copper citrate and copper azole, may still be use to treat wood destined for residential use. However, existing structures built from CCA-treated wood are not affected by this action, and CCA-treated wood can still be used for industrial construction.

The potential risk associated with structures built with CCA-treated wood arises from the fact that As, Cr and Cu can be leached and thus transported into the environment. In sufficient quantities, these metals could be toxic to non-target organisms. Numerous factors affect the quantities of

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As, Cr and Cu that can leach from CCA-treated wood, including the wood type and preservation method, pH and ionic strength of the leaching solution, and the length of contact between wood and leaching solution (Hingston et al., 2000; Townsend et al., 2004). However, soil near CCA-treated utility poles, fences, decks and boardwalks often contains more As, Cr and Cu than the surrounding soil (Hingston et al., 2000; Chirenje et al., 2003; Townsend et al., 2003). Stilwell and Gorny (1997) found that As concentrations were 20 times greater, on average, in surface soil (0–15 cm) under residential decks than in nearby control areas, while Cr concentrations were twice as high and Cu concentrations were 4 times higher under the decks than in control areas. Leached As and Cu tend to remain in the vicinity of the wooden structure because they are strongly adsorbed to soil surfaces, whereas Cr is more mobile and hence susceptible to transport through the soil profile (Allinson et al., 2000; Carey et al., 1996; Mao et al., 2004).

Soil microorganisms and other fauna will likely be impacted by the leaching of metals from CCA-treated wood structures into the soil. Most of the metals leached from CCA-treated wood structures are expected to adsorb quickly to soil particle surfaces, but may be desorbed into the soil solution after rainfall or irrigation events. Soluble metals are readily assimilated through the cellular membranes of many soil organisms, in concentrations that can lead to acute or chronic toxicity. When soil is ingested by soil fauna, metals on soil particle surfaces can be desorbed and assimilated in the animal's digestive tract. The normal activities of soil fauna expose them to soluble and adsorbed metals in soils, making them good indicators of metal bioavailability. Schultz et al. (2004) conducted a battery of standard ecotoxicology tests on topsoil that contained high As, Cu, Cr and Zn concentrations from old wood impregnation sites. The most sensitive test organisms were the soil invertebrates *Enchytraeus* sp., an enchytraeid worm and the springtail *Folsomia candida*. Yeates et al. (1994) studied the natural soil faunal populations in a grazed pasture that was contaminated by CCA from a wood treatment plant. Earthworms (*Lumbricus rubellus* and *Aporrectodea rosea*) were the most sensitive of all soil organisms studied, and were not usually found in sampling locations that were moderately to heavily contaminated (moderately contaminated sites contained, on average, 469 mg As kg^{-1}, 382 mg Cr kg^{-1} and 425 mg Cu kg^{-1}). Although earthworms are often used in standard ecotoxicology testing (OECD, 1984; ISO, 1996), there is relatively little information on how they respond to mixed metal contaminants such as CCA, since most studies have focused on the accumulation and toxicity of single metals to earthworms (Weltje, 1998).

The purpose of this study was to measure the bioavailability of metals from the wood preservative CCA and its most popular alternative, ACQ using a standard ecotoxicology test with the earthworm *Eisenia fetida*.

2. Methods

2.1. Soil characteristics

Soils were collected from two sites near Montreal, Québec, Canada (45°28′N, 73°45′W). They were mixed, frigid Typic Endoaquents (Humic Gleysols) classified as a Châteauguay silty clay loam and a Courval sandy loam. Soils were dug from the 0 to 15 cm layer, thoroughly mixed, sieved (<2 mm mesh), air-dried and stored at 20 °C until the beginning of the experiment. General characteristics of each soil are provided in Table 1.

2.2. Wood leachates

The ACQ- and CCA-treated wood was spruce (*Picea* sp.) lumber cut into 2 × 4 board lengths (about 5 cm × 10 cm). It was obtained within two weeks of treatment with ACQ or CCA, following industry standards, from the Stella-Jones Inc. lumbermill in Delson, Quebec, Canada. Untreated spruce lumber (2 × 4) was purchased from a local supplier (Rona Le Quincaillier Inc., Montreal, Quebec, Canada). The wood was shredded into small chips (about 2 mm × 2 mm × 2 mm) and approximately 0.6 kg of each wood type was placed into separate 20 L acid-washed glass containers. The acid-washing procedure for these and other glass containers involved washing the glassware with phosphate-free detergent, soaking the glassware with nitric acid (10–15%, v/v [volume/volume]) for about 10 min and then rinsing repeatedly with deionized water. We prepared synthetic rain water with a similar pH and ionic concentration as natural rainwater in Quebec (Sirois et al., 2000; Table 2), and added about 6 L to each container to achieve a mixture with 10% (w/v) of wood:synthetic rainwater. The mixture was stirred to inundate all wood chips and incubated at 20 °C for 4 days. Then, wood leachates were filtered (<0.2 μm under vacuum) and stored in acid-washed glass containers. This procedure is similar to US EPA Method 1312 where shredded wood was mixed with acidic leaching fluid (pH = 4.2) at a 20:1 liquid-to-solid ratio to generate wood leachates (Shibata et al., 2006). Dilutions (with synthetic rainwater) were prepared to give 12.5%, 25%, 50% and 75% of the ACQ-treated wood leachate and 25%, 50% and 75% of the CCA-treated wood leachate. A portion of the CCA-treated wood leachate was evaporated to obtain a higher metal concentration (200% CCA treatment). The metal concentrations in the original wood leachates (100% ACQ, 100% CCA, untreated wood), the diluted and the concentrated wood leachates are provided in Table 3.

Table 1
Selected properties of the soils (Typic Endoquents) used in the experiment

Parameter	Châteauguay soil	Courval soil
Collection site	Delson, Quebec	Notre Dame de l'Île Perrot, Quebec
	45°25′N 73°00′W	45°25′73°00′W
Sand (g kg^{-1})[a]	171	671
Clay (g kg^{-1})[a]	343	143
Silt (g kg^{-1})[a]	487	187
Textural class	Silty clay loam	Sandy loam
Soil pH[b]	6.8	7.8
Organic C (g kg^{-1})[c]	27.4	33.3
Total N (g kg^{-1})[c]	2.05	4.16
Total As (mg kg^{-1})[d]	107	20.0
Total Cr (mg kg^{-1})[d]	1.13	13.5
Total Cu (mg kg^{-1})[d]	45.2	12.4

[a]Particle-size analysis (Sheldrick and Wang, 1993).
[b]Soil:water extracts (1:2 soil:solution ratio) (Hendershot et al., 1993).
[c]Carlo Erba Flash EA NC Soils Analyzer (Milan, Italy).
[d]Analyzed with an AAS-graphite furnace system.

2.3. Earthworms

The earthworms used in this study, *E. fetida*, were purchased from Carolina Biological Supply Company (Burlington, North Carolina, USA) and kept in covered 37 L plastic containers at 20 °C, using peat moss as the bedding material. We added 5–10 g of Magic Worm Food™ (Magic Products, Amherst Junction, WI, USA) twice a week and also added water when necessary to maintain the bedding at 40–60% humidity.

2.4. Experimental design for earthworm reproduction test

The experiment was a completely random design with ACQ treatments, CCA treatments and a control (untreated wood leachate). About 500 g (dry weight basis) of soil mixed with 10 g of Magic Worm Food™ was placed in a 1 L glass jar (soil bulk density = 1 g cm^{-3}). Then, wood leachates were added to moisten the soil to 75% of water holding capacity (82 mL of wood leachate was added to the Châteauguay soil and 130 mL of wood leachate was added to the Courval soil). The water holding capacity of each soil was determined from water retention curves, using the porous plate method (Klute, 1986). Four replicates of the following treatments were added to the Châteauguay soil: 25%, 50% and 75% ACQ-treated wood leachate, 25%, 50%, 75%, 100% and 200% of CCA-treated wood leachate, and untreated wood leachate. The Courval soil received four replicates of these treatments: 12.5%, 25% and 75% ACQ-treated wood leachate, 25%, 50%, 75% and 200% of CCA-treated wood leachate, and untreated wood leachate. The treatments were not identical for both soils due to a limited quantity of wood leachate. All jars ($n = 68$) were covered and pre-incubated at 20 °C for 3 d before earthworms were added.

The earthworm reproduction test was conducted using the ISO (1996) standard method. We selected earthworms weighing between 250 and 550 mg that were sexually mature (fully developed clitellum) and determined to be in good health by visual inspection. These earthworms were placed in 1 L glass containers with Châteauguay soil or Courval soil moistened to 75% of water holding capacity with deionized water. After a two to three day acclimatization period, the earthworms were removed from the containers, surface rinsed with deionized water, and groups of ten earthworms were selected randomly, weighed and then placed in jars containing the same soil. Jars were covered with a geotextile square and a perforated lid and placed randomly in a Conviron incubator at 20 ± 1 °C with 80% humidity and a light:dark cycle of 16:8. Each week, the soil moisture was checked by weighing the jars and replenished by adding deionized water, if necessary, and jar placement inside the incubator was re-randomized.

After 28 days, sexually mature earthworms were removed from each jar and counted. Surviving *E. fetida* were placed on moistened filter paper for 24 h to void their gut, then were weighed, killed by freezing and stored at −20 °C for metal analysis. The instantaneous growth rate (IGR, g final mass g^{-1} initial mass day^{-1}) was calculated using Eq. (1):

$$\mathrm{IGR} = \ln\left(W_f/W_i\right)/\Delta t, \tag{1}$$

where W_i and W_f are initial and final earthworm mass (g), respectively, and Δt is the growth interval measured in days (Brafield and Llewellyn, 1982). After 56 days, each jar was immersed in a water bath and heated gradually from 20 to 60 °C to retrieve juvenile earthworms, and then wet sieved to collect hatched and unhatched cocoons. Cocoon hatchability (%) was the percentage of hatched cocoons amongst the cocoons collected. The number of juveniles per hatched cocoons was also calculated. A 50 g subsample of cocoon-free soil from each jar was dried (45 °C for 48 h) and retained for metal analysis.

2.5. Metal analysis

Metal concentrations in the wood leachates were determined by ICP-OES (Varian Inc., California, USA, Table 3). The metal concentration in soil was determined using the standard USEPA Method 3052 (USEPA, 1996). Briefly, about 0.25 g of dry soil was placed in a Teflon digestion vessel with 9 mL of concentrated HNO_3 (trace metal grade) and 3 mL of concentrated HCl (trace metal grade), then digested in an ETHOS-Plus microwave system (Milestone Inc., Shelton, CT, USA) for 25 min. After cooling to room temperature (20 °C), samples were diluted to 50 mL with deionized water and the As, Cr and Cu concentrations were analyzed using an atomic absorption spectrometer (AAS) equipped with a graphite furnace (Hitatchi Z8200 Polarized Zeeman atomic absorption spectrophotometer, Tokyo, Japan). Three to five of the adult earthworms recovered from each jar were dried (45 °C for 48 h), weighed and then placed in a Pyrex digestion vessel with 4 mL of concentrated HNO_3 (trace metal grade). The vessel sat at room temperature for 16–20 h, and was then heated to approximately 140 °C for 4–6 h (Spurgeon and Hopkin, 1996). After cooling, samples were diluted to 50 mL with deionized water and the As, Cr and Cu concentrations were analyzed with the AAS-graphite furnace system described above. Metal concentrations in soil and earthworm tissue were expressed on a mg kg^{-1} dry weight (soil or tissue) basis. The bioaccumulation factor for As, Cr and Cu (BF_{AS}, BF_{CR} and BF_{CU}, respectively) was the ratio of metals in earthworm tissue: soil for each experimental treatment.

For quality control and quality assurance purposes, we included a blank and the SS-2 certified reference soil (SCP Science, Baie d'Urfe, Quebec, Canada) with every set of digested samples. The SS-2 soil is reported to contain 78 mg As kg^{-1}, 58 mg Cr kg^{-1}, and 198 mg Cu kg^{-1}, in the same range as our samples. Soil is a more complex material (contains organic and inorganic components) than biological tissue, making it an appropriate reference material to evaluate the digestion and recovery of

Table 2
Chemical composition of the synthetic rain water used to leach metals from wood

Ion	mg L^{-1}
SO_4^{2-}	2.24
NO_3^-	1.92
Cl^-	0.13
Ca^{2+}	0.12
NH^+	0.343
Mg^{2+}	0.02
Na^+	0.04
K^+	0.02
pH = 4.22	

Table 3
Metal concentrations (mg L^{-1}) in leachate from ACQ-treated wood, CCA-treated wood and untreated wood. Values are the mean (and standard deviation) of 4 analytical replicates

Leachate	Al	As	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	S	Zn
ACQ-treated wood	0.23 (0.4)	0.67 (1.2)	67 (0.6)	ND[a]	ND	0.93 (0.1)	831 (19)	4.2 (0.3)	13 (0.5)	4.3 (0.3)	ND	ND	1112 (32)	0.67 (1.2)
CCA-treated wood	0.33 (0.6)	325 (5)	101 (1.5)	ND	ND	291 (3.4)	248 (4.2)	15 (0.3)	17 (0.2)	12 (0.3)	ND	ND	1096 (7.3)	2.7 (2.5)
Untreated wood	0	0.5 (0.7)	131 (1.0)	ND	ND	0.35 (0.5)	0.55 (0.8)	1.3 (1.8)	11 (2.3)	3.4 (4.7)	ND	ND	639 (7.4)	0.5 (0.7)

[a]ND, not detected with ICP-OES system.

As, Cr and Cu from soils and earthworm tissue. We found an average of 86 mg As kg^{-1}, 54 mg Cr kg^{-1}, and 203 mg Cu kg^{-1} in digested SS-2 soil ($n = 9$), which is within the 95% confidence interval reported by SCP Science.

2.6. Statistical analysis

Prior to analysis, the data were tested for normality using the Kolmogorov–Smirnov test with the PROC UNIVARIATE function of SAS (SAS System 9.1, SAS Institute Inc., Cary, NC) and were $\log_e$- or arcsin square root-transformed as required to adjust for normality and stabilize variance. For each of the soils tested, the effect of wood leachates on earthworm survival, growth and reproduction was analyzed by one-way analysis of variance (ANOVA) using the PROC GLM function of SAS. Earthworm parameters affected significantly ($P<0.05$) by the leachate treatments were then evaluated with a Tukey's multiple range test at the 95% confidence level. The relationship between earthworm physiological responses (growth, reproduction) and metal concentrations in soil and earthworm tissue was evaluated with multiple linear regressions using the PROC STEPWISE function of SAS. Simple linear regressions were fitted with the SAS INSIGHT function. Values in the tables and figures are the untransformed means, with standard deviation or standard errors.

Table 4
Copper concentrations in soil and tissue of the earthworm *E. fetida* following 28-d exposure to leachate from ACQ-treated wood

Leachate treatment	Soil Cu (mg Cu kg^{-1})	Tissue Cu (mg Cu kg^{-1})	Bioaccumulation factor (BF_{CU})
Châteauguay soil			
ACQ 25%	29±4.4[a]	28±1.2	0.99
ACQ 50%	57±4.4	46±9.3	0.81
ACQ 75%	90±8.9	53±7.6	0.58
Courval soil			
ACQ 12.5%	13±3.0	4.5±1.3	0.36
ACQ 25%	45±9.3	5.1±0.4	0.11
ACQ 75%	73±5.4	19±0.5	0.26

[a]Values (mean±standard error, $n = 4$) were corrected for background levels of Cu in soil and tissue, using data from jars receiving leachate from untreated wood.

3. Results

The Cu concentration increased in adult *E. fetida* exposed to soils with leachate from ACQ-treated wood, with bioaccumulation factors of 0.58–0.99 in the Châteauguay soil and 0.11–0.36 in the Courval soil (Table 4). Earthworm survival in the ACQ treatments ranged from 95% to 100%, suggesting that the concentrations of Cu bioaccumulated were not lethal (Tables 5 and 6). The growth rate of *E. fetida*, number of cocoons produced and cocoon hatchability were not affected by exposure to the leachate of ACQ-treated wood, compared to leachate from untreated wood (Tables 5 and 6). In the Châteauguay soil, the 25% ACQ treatment yielded more juveniles and more juveniles per hatched cocoon than the control (untreated wood), but these indicators of earthworm reproductive fitness were not affected by the ACQ treatments applied to the Courval soil (Tables 5 and 6).

Adult *E. fetida* exposed to leachate from CCA-treated wood for 28 days accumulated as much as 16 mg As kg^{-1}, 49 mg Cr kg^{-1} and 16 mg Cu kg^{-1} in their tissue (Table 7). Bioaccumulation factors varied among the CCA treatments and the soils used for this study, and few consistent trends were observed. The BF_{AS} ranged from 0.03 to 0.90, while the BF_{CR} was 0.36–2.41 and the BF_{CU} was between 0.14 and 1.51 (Table 7). Yet, the metal concentrations in leachate from CCA-treated wood were considered to be not lethal, since survivorship of adult *E. fetida* was between 80% and 100% (Tables 5 and 6). There was no difference

Table 5
Survival and growth of the earthworm *E. fetida* after 28-day exposure to leachate from ACQ-treated wood, CCA-treated wood and untreated wood in the Châteauguay soil. Cocoon production and hatchability, and juvenile numbers were assessed after 56-day exposure to leachate treatments. Values are the mean (±standard error) of 4 replicates, except for CCA 25% treatment where $n = 3$

Leachate	Adult survival (%)	Adult growth ($\times 10^{-3}$ g g^{-1} day^{-1})	Number of cocoons	Cocoon hatchability (%)	Number of juveniles	Juveniles per hatched cocoon
ACQ-treated wood						
25%	100[a]	7.8±0.7a	56±5ab	94±2	116±19ab	2.4±0.5a
50%	100	5.4±0.5ab	50±4b	97±1	78±8bc	1.7±0.2ab
75%	100	4.4±1.4ab	50±3b	95±2	75±8bc	1.6±0.1ab
CCA-treated wood						
25%	97±3	0.2±2.9b	72±6a	95±2	130±8a	1.9±0.3ab
50%	93±7	3.3±3.1ab	65±5ab	94±1	133±13a	2.2±0.1a
75%	100	8.7±0.4a	52±4ab	97±1	103±12ab	2.0±0.1ab
100%	98±2	6.9±0.7ab	54±5ab	98±2	128±12a	2.5±0.2a
200%	98±2	7.8±0.9a	52±5ab	95±1	79±13bc	1.6±0.1ab
Untreated wood	95±3	2.3±1.2ab	49±3b	97±3	51±4c	1.1±0.1b
ANOVA, $P>Fr$[a]	NS	$P = 0.0091$	$P = 0.0282$	NS	$P = 0.0001$	$P = 0.0092$

[a]When one-way ANOVA proved significant ($P<0.05$), values were compared with Tukey's multiple range test at the 95% confidence level. Within a column, values with the same letter are not statistically different.

Table 6
Survival and growth of the earthworm *E. fetida* after 28-day exposure to leachate from ACQ-treated wood, CCA-treated wood and untreated wood in the Courval soil. Cocoon production and hatchability, and juvenile numbers were assessed after 56-day exposure to leachate treatments. Values are the mean (±standard error) of 4 replicates

Leachate	Adult survival (%)	Adult growth ($\times 10^{-3}$ g g^{-1} day^{-1})	Number of cocoons	Cocoon hatchability (%)	Number of juveniles	Juveniles per hatched cocoon
ACQ-treated wood						
12.5%	98±2[a]ab	20±1.8ab	54±3ab	11±2	23±5	3.8±0.6
25%	95±3ab	21±0.8ab	35±1ab	8±3	0	0
75%	98±2ab	21±1.6ab	40±9ab	19±10	0	0
CCA-treated wood						
25%	98±2ab	21±1.0ab	82±9a	4±2	6±4	6.3±5.1
50%	98±2ab	21±2.1ab	66±9a	6±2	2±1	0.2±0.1
75%	85±3bc	15±2.8b	28±9b	2±1	0	0
200%	80±4c	1.3±1.3c	2±1c	0	0	0
Untreated wood	100a	24±0.4a	58±14ab	15±7	31±20	2.3±0.5
ANOVA, $P>$Fr[a]	$P=0.0006$	$P=0.0001$	$P=0.0001$	NS	NS	NS

[a]When one-way ANOVA proved significant ($P<0.05$), values were compared with Tukey's multiple range test at the 95% confidence level. Within a column, values with the same letter are not statistically different.

Table 7
Arsenic, chromium and copper concentrations in soil and tissue of the earthworm *E. fetida* following 28-day exposure to leachate from CCA-treated wood, and bioaccumulation factors (BF_{AS}, BF_{CR} and BF_{CU}, respectively)

Leachate treatment	Soil As (mg As kg^{-1})	Tissue As (mg As kg^{-1})	BF_{AS}	Soil Cr (mg Cr kg^{-1})	Tissue Cr (mg Cr kg^{-1})	BF_{CR}	Soil Cu (mg Cu kg^{-1})	Tissue Cu (mg Cu kg^{-1})	BF_{CU}
Châteauguay soil									
CCA 25%	7.9±2.5[a]	5.2±3.0	0.66	24±3.1	20±3.2	0.83	12±2.5	2.7±1.0	0.23
CCA 50%	8.0±4.3	7.2±1.4	0.90	27±4.0	34±6.5	1.26	5.5±3.7	8.3±1.6	1.51
CCA 75%	25±5.0	2.8±1.4	0.11	40±1.8	38±9.1	0.95	11±4.1	9.5±2.3	0.86
CCA 100%	32±5.4	9.4±2.8	0.29	49±3.7	31±7.0	0.63	16±4.4	16±3.9	1.00
CCA 200%	73±7.3	16±2.1	0.22	89±6.4	32±7.7	0.36	65±8.9	15±4.3	0.23
Courval soil									
CCA 25%	12±1.1	1.7±0.4	0.14	5.8±2.3	14±0.6	2.41	0	2.7±0.2	—
CCA 50%	22±1.9	5.7±0.5	0.26	23±2.1	25±1.4	1.09	6.4±2.2	4.7±0.7	0.73
CCA 75%	37±9.6	4.7±1.9	0.13	29±2.9	38±2.7	1.31	20±8.4	6.2±1.8	0.31
CCA 200%	80±11	2.1±0.7	0.03	79±8.4	49±6.7	0.62	58±9.8	8.3±1.5	0.14

[a]Values (mean±standard error, $n=4$) were corrected for background levels of Cu in soil and tissue, using data from jars receiving leachate from untreated wood.

in *E. fetida* growth or the number of cocoons deposited in the Châteauguay soil, but more juveniles were produced in the 25%, 50%, 75% and 100% CCA treatments than the control (untreated wood), and there were more juveniles per hatched cocoon in the 50% and 100% CCA treatments than the control (Table 5). In the Courval soil, *E. fetida* growth was significantly ($P<0.05$, Tukey test) lower in the 75% CCA and 200% CCA treatments than the control, and there were fewer cocoons in the 200% CCA treatment than the control, but other measures of earthworm reproduction were unaffected when *E. fetida* were exposed to leachate from CCA-treated wood (Table 6).

Multiple regression equations fitted using a stepwise procedure indicated that the tissue Cu concentration of adult *E. fetida*, exposed to leachate from ACQ-treated wood for 28 days, was a significant predictor of adult growth, cocoon hatchability and the number of juveniles recovered from experimental jars (Table 8a). Cocoon hatchability and the number of juveniles per hatched cocoon were also related to the soil Cu concentration (Table 8a). In general, *E. fetida* growth decreased as the tissue Cu concentration increased, whilst the cocoon hatchability and number of juveniles increased as the tissue Cu concentration increased (Fig. 1). The R^2 of the simple linear regressions describing these relationships ranged from 0.29 to 0.67.

In contrast, *E. fetida* growth and reproduction was not consistently predicted by any one metal found in soil or earthworm tissue in jars where leachate of CCA-treated wood was applied (Table 8b). The lack of clear

Table 8
Parameter estimates and coefficients of determination (R^2) for the relationships between *E. fetida* physiological responses (growth, reproduction), and metal concentrations in soil and tissue of adult *E. fetida* following 28-day exposure to leachate from a) ACQ-treated wood and b) CCA-treated wood

Earthworm response	Multiple linear regression	R^2
(a) *Data were pooled amongst soils* ($n = 24$)		
Adult growth	−0.30 (tissue Cu)***	0.72
Number of cocoons	NS	NS
Cocoon hatchability	1.53 (tissue Cu)** -0.60 (soil Cu)#	0.39
Number of juveniles	0.16 (tissue Cu)***	0.69
Juveniles per hatched cocoon	−0.028 (soil Cu)**	0.34
(b) *Data were pooled amongst soils* ($n = 35$)		
Adult growth	NS	NS
Number of cocoons	−0.037 (tissue Cr)*	0.16
Cocoon hatchability	0.039 (soil Cr)* + 0.036 (soil As)***	0.67
Number of juveniles	NS	NS
Juveniles per hatched cocoon	0.080 (soil Cr)*** + 0.034 (tissue Cu)#	0.61

Significant at #$P<0.1$, *$P<0.05$, **$P<0.01$ and ***$P<0.001$, or not significant (NS).

relationships is probably related to the disparate behavior of earthworms in the two soils studied. The growth rate of *E. fetida* was higher in the Courval soil than the Châteauguay soil. However, earthworms in the Courval soil grew less quickly when they were exposed to more concentrated leachates and accumulated more As in their tissue, while earthworms in the Châteauguay soil tended to grow more rapidly under these same conditions (Fig. 2). Despite their high growth rates, earthworms in the Courval soil laid very few cocoons and produced few offspring, while earthworms in the Châteauguay soil produced between 52 and 72 cocoons per jar, which hatched to yield 79–133 juveniles per jar (Tables 5 and 6, Fig. 2).

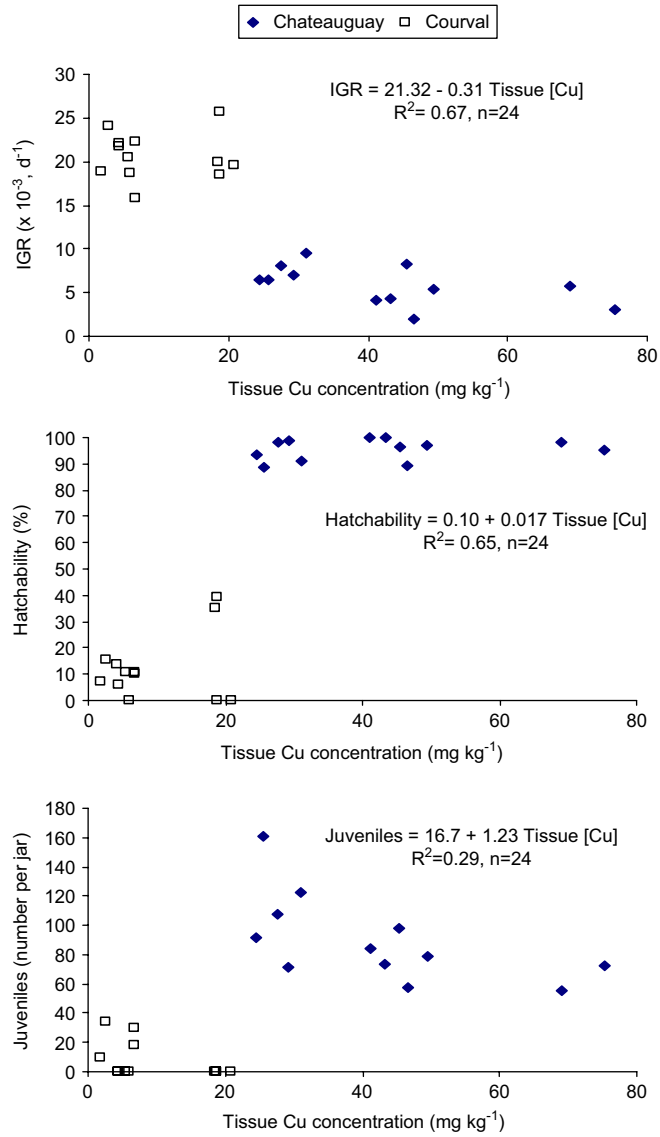

Fig. 1. Growth of *E. fetida*, hatchability and number of juveniles produced from their cocoons related to the tissue Cu concentration of adults exposed leachate of ACQ-treated wood.

4. Discussion

The bioaccumulation of As, Cr and Cu by *E. fetida* can impair growth, affect reproduction and cause death. Fischer and Koszorus (1992) reported the 56-day LC_{50} was 100 mg potassium arsenate kg^{-1} wet weight in a substrate composed of peaty marshland soil mixed with horse manure, but this dose was not toxic to *E. fetida* cultured in artificial soil (70% sand, 20% kaolin clay and 10% *Sphagnum* peat adjusted to pH 6 with $CaCO_3$) (Lock and Janssen, 2002a). In artificial soil, the no-observed effects concentration was 10 mg As kg^{-1} (all weights expressed on a dry weight basis, unless otherwise indicated) and the least-observed effects concentrations for *E. fetida* was 18 mg As kg^{-1} (Lock and Janssen, 2002a). Chromium toxicity is affected by the chemical form, and chromate, Cr (III), tends to be less toxic than dichromate, Cr (VI). Sivakumar and Subbhuraam (2005) evaluated the toxic effects of chromium exposure on *E. fetida*, and found 14-day LC_{50} values ranging from 1656 to 1902 mg kg^{-1} for Cr (III) and from 222 to 257 mg kg^{-1} for Cr (VI) in ten soils. Cocoon production by *E. fetida* was reduced by 50% after exposure to between 679 and 1110 mg Cr (III) kg^{-1} (Lock and Janssen, 2002b). Cocoon production by *E. fetida* was reduced by 50% in a sandy soil containing 210 mg Cu kg^{-1} (Scott-Fordsmand et al., 2000). Applying leachate from ACQ- and CCA-treated wood increased soil metal concentrations, but the highest measured levels in earthworm tissue were 80 mg As kg^{-1}, 89 mg Cr kg^{-1} and 90 mg Cu kg^{-1}, all of which appeared to be not lethal based on data from the scientific literature and *E. fetida* survival in this study. We did not investigate the metal speciation in soils or earthworm tissue, and this merits further research.

It is well documented that soil characteristics such as pH, clay content, organic matter content and cation exchange

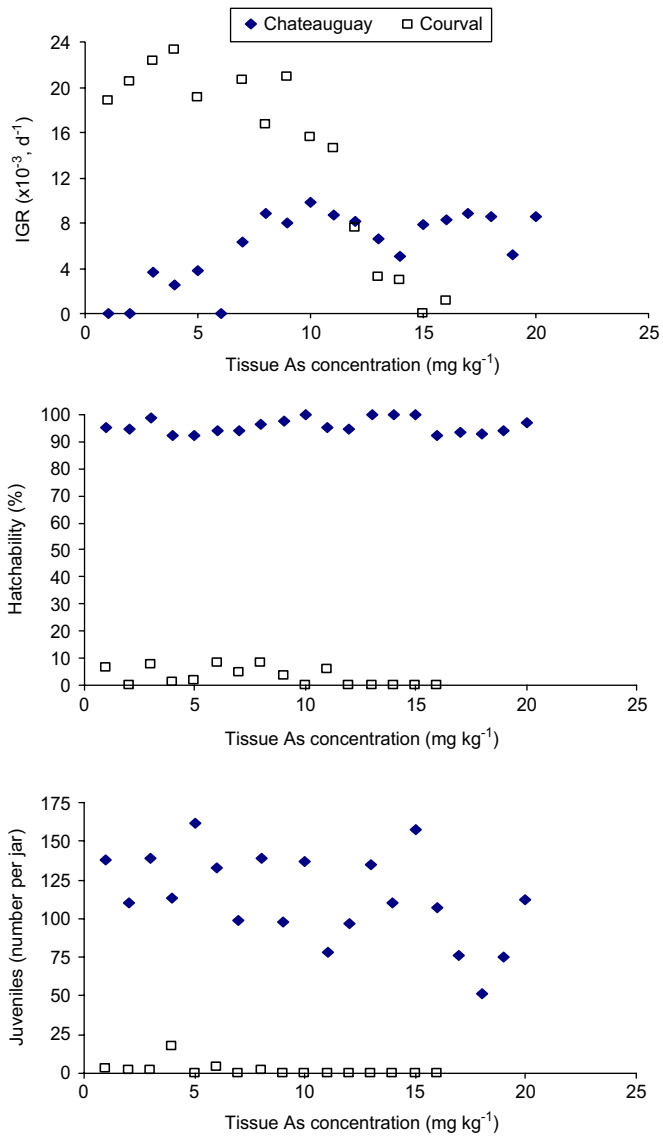

Fig. 2. Growth of *E. fetida*, hatchability and number of juveniles produced from their cocoons related to the tissue Ar concentration of adults exposed leachate of CCA-treated wood.

capacity affect the bioavailability of metals and hence their toxicity to *E. fetida* and other soil invertebrates. Metal bioavailability often declines as co-precipitates form in soil, but it can take some time for this aging effect to occur (Martinez and McBride, 2001). In this short-term study, Cu bioaccumulation by *E. fetida* exposed to leachate from ACQ-treated wood was greater in the Châteauguay soil (pH 6.8) than the Courval soil (pH 7.8), which is consistent with other reports that cite soil pH as the main factor controlling Cu bioavailability (Daoust et al., 2006; Sauvé et al., 2000). Although growth rates declined as the Cu concentration in *E. fetida* tissue increased, there was an increase in the number of hatched cocoons and juveniles produced with an increasing tissue Cu concentration. Helling et al. (2000) reported an increase in reproductive success (number of hatchlings per total number of cocoons) of *E. fetida* when substrate contamination increased from 4.02 to 8.92 mg Cu kg^{-1} wet weight, followed by a significant decline in reproductive success at higher contaminant loads.

One difficulty in assessing toxicological responses in this study was the different patterns of earthworm growth and reproduction in the soils selected. We observed faster growth of *E. fetida* in the Courval soil than the Châteauguay soil, although the average initial weight of adult earthworms was similar (0.318 ± 0.019 SD in the Châteauguay soil, 0.339 ± 0.014 SD in the Courval soil). These results suggest that the Courval soil was a more favorable environment for earthworm growth than the Châteauguay soil. The Courval soil had a higher soil pH, about 1.2 times more soil organic C and lower natural levels of total As and total Cu than the Châteauguay soil (Table 1). Further work would be necessary to determine how these soil factors, singly or in combination, may have affected *E. fetida* growth.

Adult *E. fetida* produced, on average, 49 cocoons in the Châteauguay soil control (untreated wood leachate) and 58 cocoons in the Courval soil control, indicating that earthworm reproduction was not impacted by adult growth. Yet, the hatchability of cocoons was much less in the Courval soil (0–19%) than in the Châteauguay soil (94–97%). For *E. fetida* growing in uncontaminated substrates, Dominguez et al. (2005) reported cocoon hatchability ranged from 61.2% to 89.2%, and 2.11% to 4.55 juveniles produced per cocoon. Our results from the Châteauguay soil, but not the Courval soil, are consistent with Dominguez et al. (2005). One possible explanation for poor cocoon hatchability in the Courval soil comes from the dynamic energy budget theory (Kooijman, 2000; Nisbet et al., 2000), validated as a reference model for earthworms by Jager et al. (2006). The high growth rates of *E. fetida* in the Courval soil suggest that these earthworms allocated more energy to growth than reproduction, which could lead to lower cocoon weight and hence delay cocoon development and hatching. We did not measure cocoon weight, but this parameter could be an important indicator of earthworm reproductive success.

In summary, we found that earthworm tissue concentrations of 80 mg As kg^{-1}, 89 mg Cr kg^{-1} and 90 mg Cu kg^{-1} were not lethal to *E. fetida*. There was Cu bioaccumulation in *E. fetida* exposed to soil contaminated with a single metal (Cu from ACQ-treated wood), but Cu was less bioavailable in alkaline than neutral soil conditions. Exposure to Cu alone did not affect *E. fetida* growth or reproduction, but exposure to multiple metals (As, Cr and Cu from CCA-treated wood) produced soil-specific growth and reproduction outcomes. We conclude that *E. fetida* growth rates and reproductive responses can be affected significantly by the type of soil used. Further work is needed to determine what soil factors may induce changes in energy allocation and cocoon development by *E. fetida*, as such behavior may affect the interpretation of results from the earthworm ecotoxicology test.

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