

This is a self-archived version of an original article. This version may differ from the original in pagination and typographic details.

Author(s): Kilpi-Koski, Johanna; Penttinen, Olli-Pekka; Väisänen, Ari O.; van Gestel, Cornelis A. M.

Title: Toxicity of binary mixtures of Cu, Cr and As to the earthworm *Eisenia andrei*

Year: 2020

Version: Published version

Copyright: © The Author(s) 2020

Rights: CC BY 4.0

Rights url: <https://creativecommons.org/licenses/by/4.0/>

Please cite the original version:

Kilpi-Koski, J., Penttinen, O.-P., Väisänen, A. O., & van Gestel, C. A. M. (2020). Toxicity of binary mixtures of Cu, Cr and As to the earthworm *Eisenia andrei*. *Ecotoxicology*, 29(7), 900-911.
<https://doi.org/10.1007/s10646-020-02240-1>



Toxicity of binary mixtures of Cu, Cr and As to the earthworm *Eisenia andrei*

Johanna Kilpi-Koski¹ · Olli-Pekka Penttinen¹ · Ari O. Väisänen² · Cornelis A. M. van Gestel³

Accepted: 10 June 2020
© The Author(s) 2020

Abstract

Chromated copper arsenate (CCA) mixtures were used in the past for wood preservation, leading to large scale soil contamination. This study aimed at contributing to the risk assessment of CCA-contaminated soils by assessing the toxicity of binary mixtures of copper, chromium and arsenic to the earthworm *Eisenia andrei* in OECD artificial soil. Mixture effects were related to reference models of Concentration Addition (CA) and Independent Action (IA) using the MIXTOX model, with effects being related to total and available (H₂O and 0.01 M CaCl₂ extractable) concentrations in the soil. Since only in mixtures with arsenic dose-related mortality occurred (LC₅₀ 92.5 mg/kg dry soil), it was not possible to analyze the mixture effects on earthworm survival with the MIXTOX model. EC₅₀s for effects of Cu, Cr and As on earthworm reproduction, based on total soil concentrations, were 154, 449 and 9.1 mg/kg dry soil, respectively. Effects of mixtures were mainly antagonistic when related to the CA model but additive related to the IA model. This was the case when mixture effects were based on total and H₂O-extractable concentrations; when based on CaCl₂-extractable concentrations effects mainly were additive related to the CA model except for the Cr–As mixture which acted antagonistically. These results suggest that the CCA components do interact leading to a reduced toxicity when present in a mixture.

Keywords Mixture toxicity · CCA metals · Bioavailability · MIXTOX model · Freundlich sorption isotherms

Introduction

Following over 200 years of industrialization, soil contamination is a widespread problem in many countries. According to data collected through a European Network, mineral oil and metals are the main contaminants contributing 60% to soil contamination in Europe (Panagos et al. 2013). Especially metals and persistent organic

compounds can still be found at high concentrations decades after the emissions have ceased (Hagner et al. 2017). Metals enter the environment through different anthropogenic sources like mining, traffic, smelters and the emission of combustion by-products.

Finland has around 24,000 contaminated sites, including 200 sites used for wood salt impregnation (Pyy et al. 2013) polluted by the inorganic wood impregnation chemical chromated copper arsenate (CCA) posing a high environmental risk (Karjalainen et al. 2009). The need for a risk assessment of CCA-contaminated soils is evident because contaminants have complex relationships with the natural hydrogeochemical environment and biota. For example, the reduced abundance of decomposer organisms and the effects observed in invertebrate toxicity tests imply that risks to the soil ecosystem do occur. However, one of the major conclusions of an earlier study was that to accurately assess the interactions of the metals in CCA-contaminated soils and to determine the metal(s) most contributing to the actual risk at wood treatment sites, more tests are needed both on the single metals and their mixtures (Karjalainen et al. 2009).

Supplementary information The online version of this article (<https://doi.org/10.1007/s10646-020-02240-1>) contains supplementary material, which is available to authorized users.

✉ Johanna Kilpi-Koski
Johanna.kilpi-koski@laded.fi

¹ Department of Environmental Sciences, Faculty of Biological and Environmental Sciences, University of Helsinki, Niemenkatu 73, 15140 Lahti, Finland

² Department of Chemistry, University of Jyväskylä, PL 35, 40014 University of Jyväskylä, Finland

³ Department of Ecological Science, Faculty of Science, Vrije Universiteit, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

Some studies dealt with the distribution and mobility of the CCA metals in soil (Balasoïu et al. 2001), and the toxicity to soil invertebrates of the single metals is generally well known (Peijnenburg et al. 1999b; Spurgeon and Hopkin 1996; Leduc et al. 2008; Meharg et al. 1998; Nahmani et al. 2007). The complexity and the challenges of metal toxicity in soil have recently been summarized by Moyson et al. (2018) who stated that the effects of metal mixtures are not yet well understood and that there is a notable paucity of information on their effects on soil invertebrates. The interaction effects are dependent on the metal combinations employed (Moyson et al. 2018). Interactions may occur at different steps of the intoxication process, like sorption to the soil determining metal availability, the uptake in the organism determining body concentrations and the interaction with the target site determining toxicity (Van Gestel and Hensbergen 1997). Therefore, information regarding interaction patterns of the CCA metals is highly relevant. Such interactions, however, have not been investigated so far.

In earthworms, Cu is mainly regulated by the metallothionein protein (Fisker et al. 2011). Chromium and copper showed similar fast uptake and elimination patterns in the earthworm *Eisenia andrei*, while arsenic uptake and elimination was slow, not reaching steady state within 3 weeks (Kilpi-Koski et al. 2019). These differences in uptake kinetics cause that effects of exposure to mixtures of the CCA metals are hard to predict. To assess the potential risk of metal pollution to terrestrial ecosystems, mixture toxicity tests are needed because the single metals may interact, potentially leading to higher or lower toxicity of the mixture than expected (Van Gestel et al. 2011).

In this present study we exposed the earthworm *Eisenia andrei*, as a representative of an important and ecologically relevant group of soil invertebrates, to binary mixtures of Cu, Cr and As. We determined deviations of mixture toxicity from the reference models of concentration addition (CA) and independent action (IA) (Gomez-Eyles et al. 2009; Van Gestel et al. 2011) by applying the MIXTOX model of Jonker et al. (2005). Toxicity tests were performed simultaneously with the individual metals and the binary mixtures at various concentration ratios. Mixture effects were compared to effects of the single metal, and related to total and available concentrations in the soil.

Our hypotheses were that As, Cr and Cu in binary mixtures would be more toxic than the single metals alone in affecting earthworm survival and reproduction. This assumption of synergistic interactions between the CCA metals is based on the dissimilarity in their modes of action and the differences in toxicokinetics in *E. andrei* found in our earlier study (Kilpi-Koski et al. 2019).

Materials and methods

Earthworms

Earthworms *Eisenia andrei* have been cultured at the Vrije Universiteit, Amsterdam, The Netherlands for many years. The earthworm cultures were fed with horse dung, free of pharmaceuticals or any other contaminants. For the tests, only adult earthworms with a well-developed clitellum were used. Before use in the tests, the earthworms were acclimatized in OECD artificial soil (OECD 1984) for 24 h at 20 ± 1 °C and a light:dark cycle of 16/8 h.

Preparation of the test soils

The earthworms were exposed in OECD artificial soil (OECD 1984). The artificial soil contained 10% <2 mm sphagnum peat, 70% <1 mm quartz sand and 20% kaolin clay and 0.4% CaCO₃, on a dry weight basis, which were thoroughly mixed using a household mixer. After mixing, pH-H₂O was 6.27 and pH-CaCl₂ 5.79. Soil moisture content was brought to 71% (w/w), which corresponds with 50% of the water holding capacity (WHC).

Stock solutions of K₂CrO₄ (Sigma-Aldrich ≥99.0%), CuCl₂·2H₂O (Sigma-Aldrich ≥99.0%) and Na₂HAsO₄·7H₂O (Sigma-Aldrich ≥99.0%) in water were used to spike the artificial soil. In this way, water content of the soil was adjusted to the right level when introducing the metals.

Three binary mixtures (copper-arsenic, copper-chromium and chromium-arsenic) were studied. The experimental design for the binary mixture experiment was based on the toxic unit (TU) concept with reproduction as the endpoint, with TU defined as:

$$TU = \frac{c}{EC_{50}},$$

where EC₅₀ is the median effective concentration causing 50% reduction of earthworm reproduction (in mg/kg dry soil), and c is the metal concentration in the mixture (in mg/kg dry soil).

Test concentrations chosen were based on the few available data on the toxicity of the three metals (Cu, Cr and As) to earthworms (Koster et al. 2006; Sivakumar and Subbhuraam 2005; Langdon et al. 2001a).

The mixtures tested had metal ratios of 1:1, 9:1, 1:9, 1:3 and 3:1. See Fig. S1 in the Supporting Information for the experimental design. After introduction of the metals, the artificial soil was equilibrated for three weeks before starting earthworm exposures.

Toxicity testing

Portions equivalent with 500 g dry weight artificial soil were put into 800 ml glass jars. Considering the work load

of a full mixture toxicity test and the fact that a regression-based model was used for data analysis, only three replicates (instead of four) were used for each concentration and five controls were included. At the start of the experiments, 9 earthworms (*E. andrei*) were taken from the acclimatization, rinsed in water, blotted dry on filter paper, weighed and added into each jar, together with 2 g of horse dung for food. Because of the lack of sufficient adult earthworms, tests were performed with 9 instead of 10 earthworms per test jar. The jars were loosely closed with a lid and incubated in a climate room at 20 ± 0.1 °C, 75% relative humidity and a 8/16 h light/dark cycle. Soil moisture content was checked once a week by weighing the jars and replenishing the water loss with deionized water. Additional horse dung was added if needed. After 4 weeks of incubation, survival and mass of the surviving earthworms were determined. Soil without earthworms was returned into the jars and incubated for another 4 weeks. After this period, the number of juveniles produced was determined by placing the jars in a water bath at 60 °C. Juveniles emerging to the surface were collected and counted.

Chemical analysis

About 500 mg (dw) artificial soil was weighed into 50 ml plastic bottles and 10 ml aqua regia (3:1 HCl:HNO₃) was added. The acids (HCl 36.5–38.0% and HNO₃ 69.0–70.0%) were supplied by J.T. Baker for trace metal analysis. The closed bottles were placed in an ultrasonic bath (Transsonic 820/H Elma®) for 3 × 3 min sonification at 45–50 °C. After sonification and cooling the samples were filtered (Whatman No 41.), diluted with high purity ELGA-water to a volume of 25 ml, and stored in plastic bottles for analysis with Inductively Coupled Plasma Optical Emission Spectrometry ICP-OES (Perkin Elmer Optima 4300DV). All the equipment was rinsed with acid before use. Reference materials SRM 2710 and SRM 2711, both certified by the National Institute of Standards and Technology (NIST), were included to check for the quality of the soil analysis. Recovery from certified reference sample SRM 2710 was 96% for As and 92% for Cu, and from sample SRM 2711 it was 96% for Cu. Unfortunately, no certified reference sample was available for Cr, but the high recoveries for Cu and As suggest that the spiking method used was appropriate in achieving the desired soil concentrations. The procedure has been described in more detail by Väisänen et al. (2002).

To determine water-extractable and CaCl₂-exchangeable metal concentrations, about 5 g moist soil was extracted with 50 ml H₂O or 50 ml 0.01 M CaCl₂, respectively by shaking for 2 h at 200 rpm. After settling overnight, pH was measured, and the overlaying solutions were 0.45 μm filtered and conserved with HNO₃ for analyzing extractable

metal concentrations (Smit et al. 1997). Extractable metal concentrations were analyzed by ICP-OES (Perkin Elmer Optima 4300DV).

Model and statistics

Metal partitioning in soil

To assess metal partitioning in the soils, Freundlich sorption isotherms were fitted to the measured total and extractable concentrations: Eq. 1.

$$C_s = K_f \times C_{ext}^n, \quad (1)$$

where C_s is total concentration in soil (mg/kg dry soil), C_{ext} the concentration in the H₂O or CaCl₂ extract (mg/L), K_f the Freundlich sorption constant ((L/kg)ⁿ), and n indicates the deviation from linearity. K_f and n were estimated from linear plots of log C_s versus log C_{ext} , in which control values were omitted.

Toxicity of single metals

Single metal toxicity data were fitted to logistic and, if applicable, hormetic dose–response models.

The logistic model for estimating EC₅₀ and EC₁₀ is given in Eqs. 2 and 3.

$$Y = \frac{Y_{max}}{1 + \left(\frac{c}{EC_{50}}\right)^{slope}}, \quad (2)$$

$$Y = \frac{Y_{max}}{1 + \left(\frac{10}{90}\right) \times \left(\frac{c}{EC_{10}}\right)^{slope}}. \quad (3)$$

The hormesis model (Van Ewijk and Hoekstra 1993) is presented in Eqs. 4 and 5 for EC₅₀ and EC₁₀ estimates respectively.

$$Y = \frac{Y_{max}(1 + f \times c)}{1 + (2f \times EC_{50} + 1) \times \left(\frac{c}{EC_{50}}\right)^{slope}}, \quad (4)$$

$$Y = \frac{Y_{max}(1 + f \times c)}{1 + \left(\frac{10}{90}\right) \times (2f \times EC_{10} + 1) \times \left(\frac{c}{EC_{10}}\right)^{slope}}. \quad (5)$$

In these equations, Y_{max} is the maximum response, c the exposure concentration, EC_{50} and EC_{10} the concentrations reducing the response by 50% and 10% compared to the control, f the hormesis parameter, and the slope indicates the steepness of the dose–response curve. Values for these parameters and corresponding 95% confidence intervals were obtained by using the nonlinear fitting procedure in SPSS.

Table 1 Nominal and measured total and H₂O- and 0.01 M CaCl₂-extractable Cu, Cr and As concentrations (mg/kg dry soil) of the single treatments in OECD artificial soil, determined at the beginning of the mixture toxicity experiments with *Eisenia andrei*. All measured concentrations are mean values \pm standard deviation ($n = 4$)

Total Cu				
Nominal	Actual	H ₂ O-extractable	CaCl ₂ -extractable	
0	0.40 \pm 0.28	0.27 \pm 0.00075	0.24 \pm 0.002	
50	38.1 \pm 3.52	1.90 \pm 0.08	1.96 \pm 1.11	
100	74.0 \pm 3.10	4.07 \pm 0.37	3.69 \pm 0.23	
200	161 \pm 2.86	9.92 \pm 1.01	8.67 \pm 1.12	
400	324 \pm 48.0	25.6 \pm 2.93	22.9 \pm 1.44	
800	557 \pm 194	102 \pm 4.79	72.5 \pm 39.6	
Total Cr				
0	5.07 \pm 3.53	3.90 \pm 5.18	0.12 \pm 0.004	
38.4	30.0 \pm 1.91	3.28 \pm 0.32	2.70 \pm 0.47	
96	78.4 \pm 7.07	7.82 \pm 0.68	6.66 \pm 0.65	
240	196 \pm 12.6	14.0 \pm 1.68	12.4 \pm 1.39	
600	445 \pm 52.0	31.2 \pm 7.98	26.5 \pm 7.37	
1500	1224 \pm 51.0	59.3 \pm 0.50	44.2 \pm 10.6	
Total As				
0	2.18 \pm 0.81	1.03 \pm 0.02	1.36 \pm 0.05	
15.4	14.6 \pm 2.26	12.3 \pm 1.42	12.1 \pm 1.06	
38.4	33.5 \pm 4.86	33.9 \pm 3.13	32.0 \pm 2.37	
96	86.9 \pm 11.7	100 \pm 58.3	78.9 \pm 13.9	
240	232 \pm 90.3	194 \pm 16.1	177 \pm 56.3	
600	570 \pm 175	529 \pm 74.2	530 \pm 58.5	

All measured concentrations are mean values \pm standard deviation ($n = 4$)

Mixture toxicity

Mixture toxicity data were analyzed using the MIXTOX model developed by Jonker et al. (2005). This model allows for comparing observed data with mixture effects expected using the concentration addition (CA) and the independent action (IA) reference models. The model used the effects seen in the single metal exposures, which were run simultaneously with each mixture experiment (see Fig. S1), as the starting point. The model was applied for every binary mixture and for every metal pool (measured total, and water or CaCl₂ extractable concentrations) to assess mixture effects on the reproduction of *E. andrei*. It was first tested for possible deviations from the reference model. If deviations were seen, the CA and IA models were extended with deviation functions including extra parameters to describe synergistic/antagonistic, dose-level and dose-ratio dependency (Loureiro et al. 2010). Data were fitted to the model using the solver function in Microsoft EXCEL.

Results

Metal availability

Soil pH-H₂O was 6.21–6.38 and pH-CaCl₂ 5.75–5.83. Actual metal concentrations were 69–81%, 74–82% and 90–97% of the nominal ones for Cu, Cr and As, respectively (Table 1). H₂O- and 0.01 M CaCl₂-extractable

concentrations were similar and increased with increasing total soil concentration (Table 1). H₂O-extractable concentrations were 5.0–18.5% of the total measured concentrations for Cu, 4.8–10.9% for Cr and 47.2–115% for As. Corresponding CaCl₂-extractable concentrations were 5.14–13.0%, 2.37–9.0% and 62.4–95.6% of the total concentration for Cu, Cr and As, respectively.

Partitioning/sorption of the metals

Tables S1–S3 (in the Supporting Information) show the Freundlich parameters for the sorption of the three metals, single and in the binary mixtures; Figs. S2–S4 compare the Freundlich K_f values for the different metal combinations. Compared to the single Cu treatment, the sorption of Cu was slightly lower at the lowest Cu:metal ratio, but higher for all other mixtures with As and Cr (Fig. S2). In the binary mixtures with Cr, there was strange outlier at Cr–Cu 50:50 but the overall trend was again a lower sorption at the 10:90 Cr–Cu ratio and increased sorption at the high Cr–Me ratios (Fig. S3). The interactions in the binary mixtures with As were more scattered, with a stronger sorption at low As–Me ratio but a lower sorption at the 30:70 As–Cr ratio that increased with increasing ratio of As–Cr (Fig. S4). Such trend was not visible for Cu.

Metal toxicity

Control survival of the earthworms was 100%, while the number of juveniles produced in the controls was

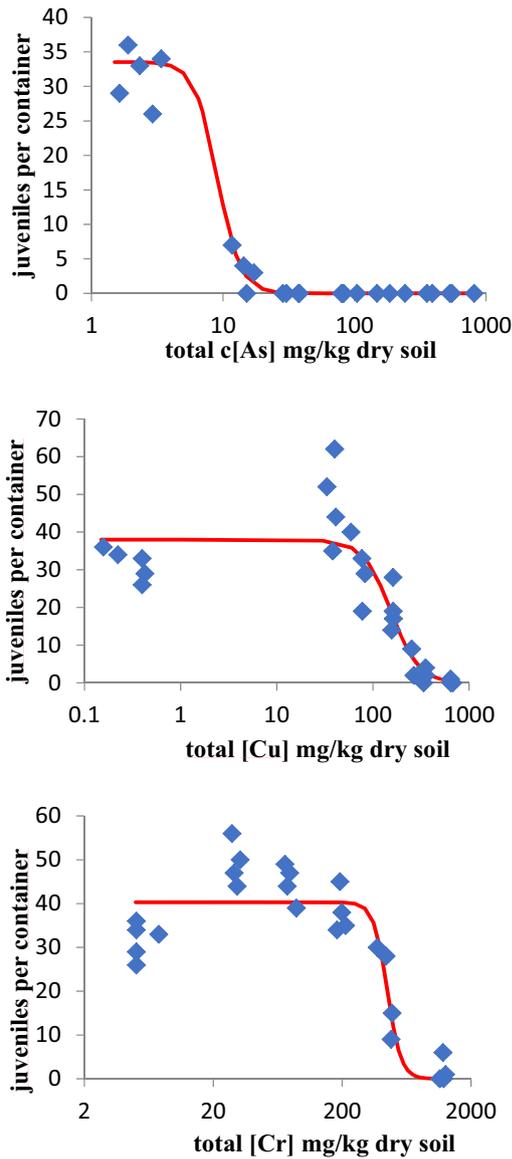


Fig. 1 Effects of the single metals As, Cu and Cr on the reproduction of *Eisenia andrei* after 8 weeks exposure in OECD artificial soils. Lines show the fit to the data of a logistic dose–response model; R^2 values for the goodness of fit of the curves were 0.981, 0.793 and 0.824 for As, Cu and Cr, respectively. See Table 2 for the EC_{50} and EC_{10} values calculated from the dose–response curves

31.6 ± 4.04 (\pm SD, $n = 5$) and coefficient of variation was 12.8%. Cu and Cr did not affect earthworm survival at the concentrations tested, but As caused a dose-related increase of mortality with an estimated LC_{50} of 92.5 (70.1–122) mg As/kg dry soil. The single metal toxicity data for effects on earthworm reproduction were reasonably well fitted using the logistic model (Eq. 2) for all three metals (Fig. 1). Hormetic effects were found at low concentrations of Cr and Cu, these data were therefore also fitted using Eq. 4 (Fig. S5). The AIC values suggested a slightly better fit of the hormetic dose–response

Table 2 EC_{50} and EC_{10} values (in mg/kg dry soil) for the effects of Cu, Cr and As on the reproduction of *Eisenia andrei* in OECD artificial soil (see Fig. 1 for corresponding dose–response curves)

Expression of exposure	Cu		Cr		As	
	EC_{50}	EC_{10}	EC_{50}	EC_{10}	EC_{50}	EC_{10}
Total concentration—logistic model	154 (106–202)	74.0 (15.1–133)	449 (399–499)	343 (215–472)	9.08 (5.96–12.2)	5.82 (1.41–10.2)
Total soil concentration—hormesis model	148 (101–194)	28.1 (5.74–50.5)	546 (279–813)	67.4 (29.0–106)	N.A.	N.A.
H ₂ O extractable	9.70 (5.62–13.8)	3.66 (–0.01 to 7.43)	26.8 (24.6–29.0)	23.7 (17.6–29.4)	5.76 (–1.60 to 13.1)	2.66 (–4.26 to 9.57)
CaCl ₂ extractable	9.01 (5.51–12.5)	3.92 (0.39–7.45)	23.9 (16.6–31.2)	11.9 (3.62–20.1)	5.96 (–3.76 to 15.7)	2.95 (–6.59 to 12.5)

In between brackets, the corresponding 95% confidence intervals are given. All values are estimated using a logistic dose–response model. For Cu and Cr, also EC_{50} and EC_{10} values based on measured total soil concentrations are included that were estimated using a hormesis model (see Fig. S4 in the Supporting Information)
N.A. not applicable

model to the Cu and Cr data than the logistic model. Both models gave similar EC_{50} values, but EC_{10} s estimated with the hormesis model were much lower (Table 2). EC_{50} s calculated with the hormesis model for Cr and Cu were 546 and 148 mg/kg in dry soil, respectively. Since the hormetic response might also be due to the fairly low control performance, for the mixture toxicity analysis the logistic model was used.

Binary mixture exposures

Only in the mixtures with arsenic, significant and dose-related mortality occurred. Since no mortality occurred in the single Cr and Cu exposures, it was not possible to analyze the mixture effects on earthworm survival using the MIXTOX model. A likelihood ratio test showed that the interaction of Cu and As was significantly antagonistic ($X^2_{(df=1)} = 5.15$, $p < 0.05$) while that of Cr and As was not ($X^2_{(df=1)} = 1.97$, n.s.).

Results of the analysis of the effects of the binary metal mixtures of Cu–As, Cu–Cr and Cr–As on the reproduction of *E. andrei* using the reference models of concentration addition (CA) and independent action (IA) are shown Tables 3–5 when related to total metal concentrations. In the Supporting Information Tables S4–S6 and S7–S9 show mixture effects based on H₂O and 0.01 M CaCl₂ extractable concentrations, respectively. The results of all mixture toxicity calculations are summarized in Table 6.

For the Cu–As mixture, there was overall antagonism when tested against the CA model ($a = 1.96$), without further deviations. The data did fit the Independent Action model without any further deviations. The same pattern was seen when mixture effects were related to

H₂O-extractable concentrations. When related to 0.01 M CaCl₂-extractable concentrations, mixture effects, however, were additive when analyzed using the CA model and synergistic according to the IA model (Table 3, S4 and S7).

For the Cr–Cu mixture, there was overall antagonism when tested against the CA model, with significant DR dependent deviations. The values of $a = 4.15$ and $b = -4.21$ indicate synergism when copper dominates the mixture and antagonism when chromium is the dominating element. Interactions were best explained from the Independent Action model. When related to H₂O-extractable concentrations, the Cr–Cu mixture acted antagonistic according to the CA model and additive according to the IA model, with in both cases a dose-related dependency suggesting a switch from antagonism to synergism already at concentrations far below the EC_{50} . When based on 0.01 M CaCl₂-extractable concentrations, the Cr–Cu mixture acted additively according to the CA model and synergistic according to the IA model (Table 4, S5 and S8).

For the Cr–As mixture, both the CA and the IA model indicated antagonism. The CA model showed slight DR or DL dependent deviations, which were significant only when unrealistically large slopes of the dose–response curves were allowed. These deviations therefore were not considered realistic. The IA model pointed into the direction of DR-dependent deviations; $a = 21.1$ (fixed slope) means antagonism, except for those mixture ratios where significant negative b values indicate synergism. The parameter value $b = -26.2$ (fixed slope) suggests that synergism is mainly seen when As is dominating the mixture. When related to H₂O and CaCl₂-extractable concentrations, the Cr–As mixture acted antagonistic according to both the CA and IA models (Table 5, S6 and S9).

Table 3 Effects of a binary mixture of copper (Cu)–arsenic (As) on the reproduction of *Eisenia andrei* (number of juveniles per pot) exposed for 8 weeks in spiked OECD artificial soil, based on measured total soil concentrations. Values printed in bold indicate the best model fit and were used for drawing conclusions on the toxicity of the mixture

	Total		S/A		DR		DL	
	CA	IA	CA	IA	CA	IA	CA	IA
	Max	35.6	36.5	36.0	36.7	35.9	36.8	35.5
Slope Cu	4.95	2.95	3.51	2.47	3.74	2.25	With slope fixed at 3.75	2.47
Slope As	7.46	6.97	7.98	6.05	7.50	6.16	7.50	2.45
EC_{50} Cu (mg/kg)	274	154	158	103	189	126	164	104
EC_{50} As (mg/kg)	13.3	11.7	11.2	10.5	11.0	10.3	11.2	6.59
a			1.96	3.02	2.40	5.0	2.69	30.4
b					−1.72	−5.69	0.20	0.97
$\chi^2 = p(x^2)$			3.1×10^{-5}	0.17	0.408	0.41	0.70	0.17
R^2	0.803	0.831	0.836	0.835	0.837	0.836	0.836	0.838

Values are calculated using the concentration addition model (CA) and the independent action model (IA) extended with deviation parameter a to show synergism/antagonism (S/A) and an additional deviation parameter b for dose ratio-dependent (DR) or dose level-dependent deviations (DL). The $p(X^2)$ values indicate the significance of the additional deviation parameters

Table 4 Effects of a binary mixture of copper (Cu)–chromium (Cr) on the reproduction of *Eisenia andrei* (number of juveniles per pot) exposed for 8 weeks in spiked OECD soil based on total soil concentrations. Values printed in bold indicate the best model fit and were used for drawing conclusions on the toxicity of the mixture

	Total							
			S/A		DR		DL	
	CA	IA	CA	IA	CA	IA	CA	IA
Max	41.3	40.4	40.5	40.5	39.7	40.4	40.4	41.2
Slope Cu	2.40	2.50	2.38	2.47	2.39	2.52	2.36	2.52
Slope Cr	2.04	2.68	3.16	2.44	7.37	2.54	3.16	2.25
EC ₅₀ Cu (mg/kg)	141	128	117	120	134	132	117	116
EC ₅₀ Cr (mg/kg)	596	544	495	517	450	479	496	493
<i>a</i>			2.06	0.96	4.15	4.87	2.21	0.002
<i>b</i>					-4.21	-7.27	0.04	-1290
chi = p(x ²)			0.0021	0.37	0.0436	0.16	0.914	0.41
R ²	0.764	0.782	0.786	0.784	0.795	0.790	0.786	0.786

Values are calculated based on the concentration addition model (CA) and the independent action model (IA) extended with deviation parameter *a* is showing synergism/antagonism (S/A) and an additional deviation parameter *b* for dose ratio-dependent (DR) or dose level-dependent deviations (DL). The p(X²) values indicate the significance of the additional deviation parameters

Table 5 Effects of a binary mixture of chromium (Cr)–arsenic (As) on the reproduction of *Eisenia andrei* (number of juveniles per pot) exposed for 8 weeks in spiked OECD soil based on total concentrations. Values printed in bold indicate the best model fit and were used for drawing conclusions on the toxicity of the mixture

	Total							
			S/A		DR		DL	
	CA	IA	CA	IA	CA	IA	CA	IA
Max	37.1	37.6	37.9	36.8	37.9	36.9	40.2	35.0
Slope Cr	2.27	10.5	1.54	Fixed at 2.5	1.23	Fixed at 2.5 ^a	Fixed at 1.23	Fixed at 10
Slope As	6.08	6.09	4.92	3.76	Fixed at 7.5	4.10	Fixed at 7.5	7.38
EC ₅₀ Cr (mg/kg)	1170	460	206	219	396	434 ^a	202	270
EC ₅₀ As (mg/kg)	11.2	10.5	5.98	6.8	5.77	7.09	6.24	5.80
<i>a</i>			8.10	11.8	14.1	21.1	5.64	316
<i>b</i>					-16.2	-26.2	-0.077	0.92
chi = p(x ²)			1.6 × 10⁻⁹	0.0002	0.041	0.0058	0.14	0.061
R ²	0.739	0.778	0.819	0.807	0.826	0.821	0.823	0.814

Values are calculated based on the concentration addition model (CA) and the independent action model (IA) extended with deviation parameter *a* is showing synergism/antagonism (S/A) and an additional deviation parameter *b* for dose ratio-dependent (DR) or dose level-dependent deviations (DL). The p(X²) values indicate the significance of the additional deviation parameters

^aIA model with slope of Cr fixed at 2.5 very unrealistic values for EC₅₀

Discussion

In this study, similar metal extractabilities were found for H₂O and 0.01 M CaCl₂. Freundlich parameters in some cases were influenced by metal/metal ratios. The binary mixture effects were overall antagonistic when related to the CA model and additive when related to the IA model. Deviations from this pattern were sometimes seen when relating effects to extractable concentrations.

Metal partitioning

H₂O- and 0.01 M CaCl₂-extractable concentrations were similar and increased with increasing total soil concentration. For cationic metals, like Cu, 0.01 M CaCl₂-extractable concentrations generally are higher than H₂O extractable concentrations because of cation exchange effects (Giska et al. 2014; Hobbelen et al. 2006). Sauv e et al. (1997) however, also found higher H₂O than CaCl₂ extractable

Cu concentrations in urban and agricultural soils with pH(CaCl₂) 6.99–7.62 and 6.03–7.28 and OM contents of 0.41–10.77% and 1.57–6.35%, respectively. Ca may have promoted coagulation of soluble organic matter (Sauvé et al. 1997) reducing the mobility of Cu by complexation with fulvic and humic acids (McLaren et al. 1981). Giska et al. (2014) found higher 0.01 M CaCl₂ extractable Cu fractions (0.04–0.14%) than H₂O extractable fractions (0.12–0.44%) in field-contaminated soils with a high OM content (36.3–54.2%) but lower pH-CaCl₂ (3.46–5.06) and total Cu concentrations of 27.2–67.0 mg/kg (dw). And Hobbelen et al. (2006), using field-contaminated soils with high pH-CaCl₂ (>7), OM content (15.1–30.0%) and clay content (14.7–46.3%), reported CaCl₂-extractable Cu fractions of 0.02–0.20% from the total concentrations. These extractable fractions in field soils are lower than the ones in our test (5.0–18.5%), which might be due to the lack of ageing and the use of a freshly spiked artificial soil which has a lower sorption capacity for metals.

The pH in our soil was in the range that may influence precipitation of Cu, Cr and As, making it likely they formed complexes (Kim et al. 2015; Langdon et al. 2002; Mesuere and Fish 1992). Schultz et al. (2004) found that the majority of As and Cr was less mobile because of the formation of complexes that firmly bound to clay minerals. The complexes formed may bind to soil particles (Balasoiu et al. 2001) which will have affected metal availability. Balasoiu et al. (2001) studied the fate of a commercial CCA-C-solution containing 45.5% CrO₃, 18.2% CuO and 36.3% As₂O₅ in artificial soils with different combinations of kaolinite (5–30%), sand (30–69.5%), organic matter (0.5–15%) and 25% silt. The measured pH (H₂O) was in the same range as in our study. Metal retention in the mineral artificial soil was low at 58% for Cu and at 23% for Cr but increased in high organic artificial soils to 96% for Cu and 78% for Cr. Cr is anionic metal, but its speciation form Cr(III) exists as cationic species at pH < 4 (Reijonen 2017). In soils, the mobile Cr(VI) species will be converted into the more stable Cr(III) (Kumpiene et al. 2008). Cr(III) adsorption is influenced by pH and cation exchange capacity (Choppala et al. 2010). Cr(III) was adsorbed very strongly at pH < 4 by both kaolinite and montmorillonite indicating a low mobility in soils. At pH 4–5, a combination of adsorption and precipitation processes made Cr(III) quite immobile in soil (Griffin et al. 1977). In addition, complexation with soil organic matter strongly reduces Cr(III) solubility (Reijonen 2017). As a consequence, Cr also showed low availability in our test soils, even in the absence of Fe.

The organic matter content of the soil (Feng et al. 2013) and its humic substances adsorbed onto kaolinite (Saada et al. 2003) had the greatest impact on arsenic adsorption. Balasoiu et al. (2001) pointed out that pH and oxidation

reduction are the key chemical parameters influencing As sorption. The binding of As to soil is hardly affected by Ca (Kumpiene et al. 2008). In our study As showed rather high availability, which was the same for both the H₂O and CaCl₂ extractions, and can be explained by the presence of As as an anionic species. And the high availability might also be due to the low Fe content of the artificial soil, which prohibited the formation of As–Fe complexes. The fact that the type of organic matter (peat) used to prepare the artificial soil has a different structure compared to natural organic matter probably also contributed to the high As availability.

We found that As and Cr at a 30:70 Cu:metal ratio affected the Freundlich K_f for the sorption of Cu, with n values being <1 suggesting a concentration effect. K_f values for Cu were higher when based on H₂O than 0.01 M CaCl₂ extractable concentrations (Posthuma et al. 1997). The sorption of Cu was lower at the 10:90 Cr/Cu ratio and it increased at the high Cr/Cu ratios. The sorption of As in different Cr/Me ratios was quite similar, but n values indicated possible complexation of the metals (n > 1). Cr and As can form less soluble complexes (Kües 2007). As/Cr and Cr/As ratios were studied with *Staphylococcus xylosus* (Aryal et al. 2011) showing that As(V) ion at high concentration levels suppressed the sorption of Cr. Like in our case, As sorption increased with decreasing Cr level so increasing ratio of As/Cr. This is supported by Buchter et al. (1989) who observed greater retention for As compared to Cr.

Toxicity of single metals

In our study, total soil concentrations up to 557 mg Cu/kg and 1224 mg Cr/kg did not affect the survival of *E. andrei*, but As did with an estimated LC₅₀ of 92.5 mg/kg. The dose–response curves for the toxicity to earthworm reproduction of single Cr and Cu showed hormesis, which suggests a stimulus at low doses while high doses are toxic. For Cu, deficiency might have occurred in the control, explaining for the hormetic effect at the lower concentrations added to the artificial soil. Spurgeon et al. (2004) also found hormesis for Cu when testing growth and development of juveniles of *Lumbricus rubellus*. No indications for hormetic effects of Cr on earthworms were found in the literature. A hormetic effect of As was observed for juvenile reproduction of *E. fetida* at intermediate total concentrations (<45 mg/kg, dw) in low organic mining area soils (Neaman et al. 2012; Bustos et al. 2015). In our study, for As no signs of hormesis were seen, most likely because of the strong effects on earthworm reproduction already seen at the lowest concentrations tested.

Concentrations above 100 mg Cu/kg were toxic to the earthworms *Dendrodrilus rubidus* (Savigny) and *L.*

Table 6 Summary of the results on the toxicity of binary mixtures of As, Cr and Cu to the reproduction of *Eisenia andrei* in OECD artificial soil, based on total and extractable concentrations (H₂O and 0.01 M CaCl₂)

Mixture	Expression of exposure	CA	IA
Cu–As	Total	Antagonism	Additivity
	H ₂ O extract	Antagonism	Additivity
	CaCl ₂ extract	Additivity	Synergism for S/A, no further DR and DL deviations
Cu–Cr	Total	Antagonism	Additivity
	H ₂ O extract	Antagonism; DR shows that the mixture is more antagonistic at increasing Cu concentration	Additive, but tendency to DR with antagonism at increasing Cu concentration
	CaCl ₂ extract	Additivity	Synergism for S/A, no further DR and DL deviations
Cr–As	Total	Antagonism; DR, antagonistic at increasing Cr concentration	Antagonism; DR, antagonistic increasing Cr concentration
	H ₂ O extract	Antagonism	Antagonism
	CaCl ₂ extract	Antagonism	Antagonism

Results are shown from the analyses using the concentration addition (CA) and independent action (IA) model. See Tables 3–5 and S4–S9 for the individual mixture responses

rubellus in two different field-contaminated soils with 1.58% and 10.0% organic matter and pH (H₂O) 7.18 and 5.14 (Langdon et al. 2001b). In OECD artificial soil, EC₅₀ for the effect of Cu on the growth of *E. andrei* was >100 mg/kg (dw) (Van Gestel et al. 1991). Caetano et al. (2016) reported EC₅₀ and EC₂₀ values of 130.9 and 73 mg/kg (dw) for the effects of Cu on the reproduction of *E. andrei* in a Portuguese natural soil with pH (H₂O) 5.9 and 6.5% OM. The EC₁₀ and EC₅₀ values for the toxicity of Cu to *E. fetida* in OECD artificial soil with pH (0.01 M CaCl₂) 6.5 and 4.7% organic carbon were 225 and 349 mg/kg (dw), respectively (Criel et al. 2008). The EC₅₀ and EC₁₀ values of 154 and 74 mg Cu/kg dry soil, respectively found in our study are of the same order of magnitude as the values reported in the literature for different earthworm species.

LC₅₀ values for the effects of Cr(III) and Cr(VI) on the survival of the earthworm *E. fetida* were 1656–1902 mg/kg and 222–257 mg/kg, respectively in field soil from India (Sivakumar and Subbhuraam 2005). Their soil contained only 0.14–0.68% organic carbon compared to about 5.8% in our artificial soil, explaining for the lower toxicity in our study and the absence of mortality at the highest test concentration (1500 mg/kg). Our EC₅₀ and EC₁₀ values for the effect of Cr on earthworm reproduction Cr were 449 mg/kg and 343 mg/kg. These values are in the same range as the 21-day EC₅₀ for *E. fetida* (based on cocoon production) of 892 mg Cr/kg dw reported by Lock and Janssen (2002a).

EC₅₀ and EC₁₀ values for the effects of As on the cocoon production of *E. fetida* in an agricultural soil from Chile with pH (0.1 M KNO₃) 5.7–7.6 and 0.7–5.8% OM were 22 and 8 mg/kg, respectively (Bustos et al. 2015). Lock and Janssen (2002b) reported an EC₅₀ for effects on *E. fetida* cocoon production of 10.8 mg/kg (dw) in OECD artificial

soil based on total concentration. EC₅₀ values for the toxicity of As for *E. andrei* were 56–151 mg/kg (dw) and EC₁₀ was 26 mg/kg (dw) when spiked as Na₂HAsO₄*7H₂O into soils with an OM content <10.0% and pH-H₂O 5.87–8.79 (Romero-Freire et al. 2015). The EC₅₀ and EC₁₀ obtained in our study (9.08 and 5.80 mg/kg dry soil, respectively) are in agreement with the ones reported by Bustos et al. (2015) and Lock and Janssen (2002b), but low compared to the study of Romero-Freire et al. (2015). We used the same As-salt as Romero-Freire et al. (2015) and our peat content was 10% and pH-H₂O 6.27. Soil pH influences the uptake of the As by earthworms: At pH ≤ 6.75, internal As levels increased significantly (Peijnenburg et al. 1999a). The measured pH of our spiked OECD soils was <6.75. Together with the possible low Fe content of the artificial soil and the absence of ageing this may explain for the unexpected high As toxicity to *E. andrei* in our study.

Mixture effects

Only the binary mixtures containing As showed negative, dose-related effects on the survival of *E. andrei* making it hard to interpret these mixture effects. For reproduction, antagonistic effects were found for the all binary mixtures of Cu–As, Cu–Cr and Cr–As when tested against the CA model and based on total measured soil concentrations. Using cocoon production of *E. fetida*, Spurgeon and Hopkin (1995) found antagonistic effects for mixtures of Cu, Zn, Cd and Pb. Khalil et al. (1996) reported that mixtures of Cu, Cd and Zn were antagonistic to the cocoon production of the earthworm *A. caliginosa*, also using the CA model.

When relating mixture effects to H₂O and 0.01 M CaCl₂ extractable concentrations, similar antagonistic effects in

binary mixtures of Cu–As, Cu–Cr and Cr–As on the reproduction of *E. andrei* were seen against the CA model. Dose-ratio dependent deviation was detected for H₂O and 0.01 M CaCl₂ extractable concentrations at low Cu switching to synergism in the Cu–Cr mixture and for the Cr–As mixture related to total concentrations at low Cr concentrations for both reference models of CA and IA. Related to the IA model, mainly additivity was seen when expressing effects on the basis of total and H₂O or 0.01 M CaCl₂ extractable concentrations.

Van Gestel and Hensbergen (1997) reported that interactions between Cd and Zn were antagonistic which was explained from interactions at the level of sorption to the soil, so from K_f values. This seems confirmed by the K_f values obtained in our study showing a stronger sorption when the other element was present (Table S1–S3 in the Supporting Information). This explains antagonism when relating effects to total concentrations and a shift to additivity when effects were related to H₂O and 0.01 M CaCl₂ extractable concentrations (Table 6).

The results of this study suggest that Cu, Cr and As have different modes of action as they generally showed antagonism when their mixture effect was analyzed using the CA model and additivity according to the IA model. The differences in uptake and elimination kinetics found in our earlier study (Kilpi-Koski et al. 2019) may have contributed to the rather high toxicity of As in this study and to the dissimilar action of the three metals in the mixtures.

Conclusion

In the present study we found that arsenic (As) was the most toxic metal to earthworms. Cr and Cu showed hormesis at low concentrations. Extractable concentrations showed that As had a high availability in the OECD artificial soil, which was confirmed by its high toxicity. Although availability was fairly high and did not differ between the H₂O and 0.01 M CaCl₂ extractions, the mixtures of As, Cr and Cu generally showed antagonism against the CA model for their toxicity to the reproduction of *Eisenia andrei*. This may be explained from complexation reactions in the soil but also from a different mode of action of the three metals used in chromated copper arsenate (CCA) mixtures used for wood preservation. Our results showed that CCA components interact with each other leading to a reduced toxicity in the mixtures.

Acknowledgements The Onni and Hilja Tuovinen Foundation, the Maj and Thor Nessling foundation, the Lahti Fund of University of Helsinki and the Lahti Region Development LADEC Ltd have funded this project. We thank Rudo Verweij of the Vrije Universiteit, Amsterdam for technical assistance and Virve Haili her help in performing the toxicity tests. Open access funding provided by University of Helsinki including Helsinki University Central Hospital.

Funding This study was funded by The Onni and Hilja Tuovinen Foundation, the Maj and Thor Nessling foundation, the Lahti Fund of University of Helsinki and the Lahti Region Development LADEC Ltd.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Aryal M, Ziajova M, Liakopoulou-Kyriakides M (2011) Comparison of Cr(VI) and As(V) removal in single and binary mixtures with Fe(III)-treated *Staphylococcus xylosum* biomass: thermodynamic studies. *Chem Eng J* 169:100–106
- Balasoju CF, Zagury GJ, Deschênes L (2001) Partitioning and speciation of chromium, copper, and arsenic in CCA-contaminated soils: influence of soil composition. *Sci Total Environ* 280:239–255
- Buchter B, Davidoff B, Amacher MC, Hinz C, Iskandar IK, Selim HM (1989) Correlation of Freundlich K_d and n retention parameters with soils and elements. *Soil Sci* 148:370–379
- Bustos V, Mondaca P, Verdejo J, Sauv e S, Gaete H, Celis-Diez JL, Neaman A (2015) Thresholds of arsenic toxicity to *Eisenia fetida* in field-collected agricultural soils exposed to copper mining activities in Chile. *Ecotoxicol Environ Saf* 122:448–454
- Caetano AL, Marques CR, Goncalves F, da Silva EF, Pereira R (2016) Copper toxicity in a natural reference soil: ecotoxicological data for the derivation of preliminary soil screening values. *Ecotoxicology* 25:163–177
- Choppala G, Bolan N, Mallavarapu M, Chen Z (2010) Sorption and mobility of chromium species in a range of soil types, In: 19th World Congress of Soil Science, Soil Solutions for a Changing World. Brisbane, Australia. Published on DVD
- Criel P, Lock K, van Eeckhout H, Oorts K, Smolders E, Janssen CR (2008) Influence of soil properties on Copper toxicity for two soil invertebrates. *Environ Toxicol Chem* 27:1748–1755
- Feng Q, Zhang Z, Chen Y, Liu L, Zhang Z, Chen C (2013) Adsorption and desorption characteristics of arsenic on soils: kinetics, equilibrium, and effect of Fe(OH)₃ colloid, H₂SiO₃ colloid and phosphate, 2013 International Symposium on Environmental Science and Technology (2013 ISEST). *Procedia Environ Sci* 18:26–36

- Fisker KV, Sørensen JG, Damgaard C, Pedersen KL, Holmstrup M (2011) Genetic adaptation of earthworms to copper pollution: is adaptation associated with fitness costs in *Dendrobaena octaedra*? *Ecotoxicology* 20:563–573
- Giska I, van Gestel CAM, Skip B, Laskowski R (2014) Toxicokinetics of metals in the earthworm *Lumbricus rubellus* exposed to natural polluted soils: relevance of laboratory tests to the field situation. *Environ Pollut* 190:123–132
- Gomez-Eyles JL, Svendsen C, Lister L, Martin H, Hodson ME, Spurgeon DJ (2009) Measuring and modelling mixture toxicity of imidacloprid and thiacloprid on *Caenorhabditis elegans* and *Eisenia fetida*. *Ecotoxicol Environ Saf* 72:71–79
- Griffin RA, Au AK, Frost RR (1977) Effect of pH on adsorption of chromium from landfill-leachate by clay minerals. *J Environ Sci Health* 8:431–449
- Hagner M, Romantschuk M, Penttinen OP, Egfors A, Marchand C, Augustsson A (2017) Assessing toxicity of metal contaminated soil from glassworks sites with a battery of biotests. *Sci Total Environ* 613–614:30–38
- Hobbelen PHF, Koolhaas JE, van Gestel CAM (2006) Bioaccumulation of heavy metals in the earthworms *Lumbricus rubellus* and *Aporrectodea caliginosa* in relation to total and available metal concentrations in field soils. *Environ Pollut* 144:639–646
- Jonker MJ, Svendsen C, Bedaux JJM, Bongers M, Kammenga JE (2005) Significance testing of synergistic/antagonistic, dose level-dependent, or dose ratio-dependent effects in mixture dose-response analysis. *Environ Toxicol Chem* 24:2701–2713
- Karjalainen AM, Kilpi-Koski J, Väisänen AO, Penttinen S, van Gestel CAM, Penttinen OP (2009) Ecological risks of an wood impregnation mill: application of the Triad approach. *Integr Environ Assess Manag* 5:379–389
- Khalil MA, Abdel-Lateif HA, Bayoumi BM, van Straalen NM, van Gestel CAM (1996) Effects of metals and metal mixtures on survival and cocoon production of the earthworm *Aporrectodea caliginosa*. *Pedobiologia* 40:548–556
- Kilpi-Koski J, Penttinen OP, Väisänen AO, van Gestel CAM (2019) An uptake and elimination kinetics approach to assess the bioavailability of chromium, copper, and arsenic to earthworms (*Eisenia andrei*) in contaminated field soils. *Environ Sci Pollut Res* 26:15095–15104
- Kim RY, Yoon JK, Kim TS, Yang JE, Owens G, Kim KR (2015) Bioavailability of heavy metals in soils: definitions and practical implementation—a critical review. *Environ Geochem Health* 37:1041–1061
- Koster M, de Groot A, Vijver M, Peijnenburg W (2006) Copper in the terrestrial environment: Verification of a laboratory-derived terrestrial biotic ligand model to predict earthworm mortality with toxicity observed in field soils. *Soil Biol Biochem* 38:1788–1796
- Kumpiene J, Lagerkvist A, Maurice C (2008) Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments—a review. *Waste Manag* 28:215–225
- Kües U (eds.) (2007) Wood production, wood technology, and biotechnological impacts. Universitätsverlag Göttingen, German
- Langdon CJ, Meharg AA, Feldmann J, Charnock J, Farquhar M, Pearce TG, Semple KT, Cotter-Howells J (2002) Arsenic-speciation in arsenate-tolerant and non-tolerant populations of the earthworm *Lumbricus rubellus*. *J Environ Monit* 4:603–608
- Langdon CJ, Pearce TG, Meharg AA, Semple KT (2001a) Survival and behavior of the earthworms *Lumbricus rubellus* and *Dendrodrilus rubidus* from arsenate-contaminated and non-contaminated sites. *Soil Biol Biochem* 33:1239–1244
- Langdon CJ, Pearce TG, Meharg AA, Semple KT (2001b) Resistance to copper toxicity in populations of the earthworms *Lumbricus rubellus* and *Dendrodrilus rubidus* from contaminated mine wastes. *Environ Toxicol Chem* 20:2336–2341
- Leduc F, Whalen JK, Sunahara GI (2008) Growth and reproduction of the earthworm *Eisenia fetida* after exposure to leachate from wood preservatives. *Ecotoxicol Environ Saf* 69:219–226
- Lock K, Janssen CR (2002a) Ecotoxicity of chromium (III) to *Eisenia fetida*, *Enchytraeus albidus* and *Folsomia candida*. *Ecotoxicol Environ Saf* 51:203–205
- Lock K, Janssen CR (2002b) Toxicity of arsenate to the compostworm *Eisenia fetida*, the potworm *Enchytraeus albidus* and the springtail *Folsomia candida*. *Bull Environ Contam Toxicol* 68:760–765
- Loureiro S, Svendsen C, Ferreira ALG, Pinheiro C, Ribeiro F, Soares AMVM (2010) Toxicity of three binary mixtures to *Daphnia magna*: comparing chemical modes of action and deviations from conceptual models. *Environ Toxicol Chem* 29:1716–1726
- McLaren RG, Swift RS, Williams JG (1981) The adsorption of copper by soil materials at low equilibrium solution concentrations. *J Soil Sci* 32:247–256
- Meharg AA, Shore RF, Broadgate K (1998) Edaphic factors affecting the toxicity and accumulation of arsenate in the earthworm *Lumbricus terrestris*. *Environ Toxicol Chem* 17:1124–1131
- Mesuer K, Fish W (1992) Chromate and oxalate adsorption on goethite. 2. Surface complexation modeling of competitive adsorption. *Environ Sci Technol* 26:2365–2370
- Moyson S, Vissenberg K, Fransen E, Blust R, Husson SJ (2018) Mixture effects of copper, cadmium, and zinc on mortality and behavior of *Caenorhabditis elegans*. *Environ Toxicol Chem* 37:145–159
- Nahmani J, Hodson ME, Black S (2007) Effects of metals on life cycle parameters of the earthworm *Eisenia fetida* exposed to field-contaminated, metal-polluted soils. *Environ Pollut* 149:44–58
- Neaman A, Huerta S, Sauvé S (2012) Effects of lime and compost on earthworm (*Eisenia fetida*) reproduction in copper and arsenic contaminated soils from the Puchuncaví Valley, Chile. *Ecotoxicol Environ Saf* 80:386–392
- OECD (1984) Guidelines for the testing of chemicals No. 207, Earthworm acute toxicity tests. Organisation for Economic Co-operation and Development, Paris, France
- Panagos P, Van Liedekerke M, Yigini Y, Montanarella L (2013) Review article, contaminated sites in Europe: review of the current situation based on data collected through a European Network. *J Environ Public Health* 158764:11 <https://doi.org/10.1155/2013/158764>
- Peijnenburg WJGM, Baerselman R, de Groot AC, Jager T, Posthuma L, Van Veen RPM (1999a) Relating environmental availability to bioavailability: soil-type-dependent metal accumulation in the Oligochaete *Eisenia andrei*. *Ecotoxicol Environ Saf* 44:294–310
- Peijnenburg WJGM, Posthuma L, Zweers PGP, Baerselman R (1999b) Prediction of metal bioavailability in Dutch field soils for the Oligochaete *Enchytraeus crypticus*. *Ecotoxicol Environ Saf* 43:170–186
- Posthuma L, Baerselman R, Van Veen RPM, Dirven-Van Breemen EM (1997) Single and joint toxic effects of copper and zinc on reproduction of *Enchytraeus crypticus* in relation to sorption of metals in soils. *Ecotoxicol Environ Saf* 38:108–121
- Pyy O, Haavisto T, Niskala K, Silvola M (2013) Contaminated sites in Finland—review 2013 (in Finnish). Finnish Environment Institute Reports 27/2013. The Finnish Environment Institute, Helsinki, Finland
- Reijonen I (2017) Chemical bioavailability of chromium and vanadium species in soil: risk assessment of the use of steel industry slags as liming materials. Dissertation, Department of Food and Environmental Sciences, University of Helsinki, Finland
- Romero-Freire A, Martín Peinado FJ, Díez Ortiz M, van Gestel CAM (2015) Influence of soil properties on the bioaccumulation and effects of arsenic in the earthworm *Eisenia andrei*. *Environ Sci Pollut Res* 22:15016–15028

- Saada A, Breeze D, Crouzet C, Cornu S, Baranger P (2003) Adsorption of arsenic (V) on kaolinite and on kaolinite-humic acid complexes, Role of humic acid nitrogen groups. *Chemosphere* 51:757–763
- Sauvé S, McBride MB, Norvell WA, Hendershot WH (1997) Copper solubility and speciation of in situ contaminated soils: effects of copper level, pH and organic matter. *Water Air Soil Pollut* 100:133–149
- Schultz E, Joutti A, Räisänen ML, Lintinen P, Martikainen E, Lehto O (2004) Extractability of metals and ecotoxicity of soils from two old wood impregnation sites in Finland. *Sci Total Environ* 32:71–84
- Sivakumar S, Subbhuraam CV (2005) Toxicity of chromium(III) and chromium(VI) to the earthworm *Eisenia fetida*. *Ecotoxicol Environ Saf* 62:93–98
- Smit C, van Beelen P, van Gestel CAM (1997) Development of zinc bioavailability and toxicity for springtail *Folsomia candida* in an experimentally contaminated field plot. *Environ Pollut* 98:73–80
- Spurgeon DJ, Hopkin SP (1995) Extrapolation of the laboratory-based OECD earthworm toxicity test to metal contaminated field sites. *Ecotoxicology* 4:190–205
- Spurgeon DJ, Hopkin SP (1996) Effects of metal-contaminated soils on the growth, sexual development, and early cocoon production of the earthworm *Eisenia fetida*, with particular reference to zinc. *Ecotoxicol Environ Saf* 35:86–95
- Spurgeon DJ, Svendsen C, Kille P, Morgan AJ, Weeks JM (2004) Responses of earthworms (*Lumbricus rubellus*) to copper and cadmium as determined by measurement of juvenile traits in a specifically designed test system. *Ecotoxicol Environ Saf* 57:54–64
- Väisänen A, Suontamo R, Silvonen J, Rintala J (2002) Ultrasound-assisted extraction in the determination of arsenic, cadmium, lead and silver in the contaminated soil samples by inductively coupled plasma atomic emission spectrometry. *Anal Bioanal Chem* 373:93–97
- Van Ewijk PH, Hoekstra JA (1993) Calculation of the EC50 and its confidence interval when subtoxic stimulus is present. *Ecotoxicol Environ Saf* 25:25–32
- Van Gestel CAM, Hensbergen PJ (1997) Interaction of Cd and Zn toxicity of for *Folsomia candida* Willem (Collembola: Isotomidae) in relation to bioavailability in soil. *Environ Toxicol Chem* 16:1177–1186
- Van Gestel CAM, Jonker MJ, Kammenga JE, Laskowski R, Svendsen C (Eds.) (2011) Mixture toxicity. Linking approaches from ecological and human toxicology. SETAC Press, Society of Environmental Toxicology and Chemistry, Pensacola
- Van Gestel CAM, van Dis WA, Dirven-van Breemen EM, Sparenburg PM, Baerselman R (1991) Biology and influence of cadmium, copper, and pentachlorophenol on growth and sexual development of *Eisenia andrei* (Oligochaeta; Annelida). *Biol Fert Soils* 12:117–121