Response of *Enchytraeus crypticus* worms to high metal levels in tropical soils polluted by copper smelting

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ARTICLE INFO

Article history:

Received 17 June 2013

Accepted 5 October 2013

Available online 12 October 2013

Keywords:

Soils

Copper smelting

Toxicity

Enchytraeus crypticus

Mufulira

Zambian Copperbelt

ABSTRACT

Tropical soils from the vicinity of the Mufulira copper smelter (Zambian Copperbelt) were found to contain high levels of metals, particularly in the upper parts of soil horizons. Metal concentrations varied within the ranges: 37–8980 mg Cu/kg, 3–46 mg Co/kg, <2.5–42 mg Pb/kg, and 16–83 mg Zn/kg. The EDTA extractions indicated that a considerable percentage of the metals were bound in the “labile” fractions of the soil (up to 55% of total Cu concentration, 36% Co, 41% Pb, 30% Zn). The mineralogical investigation of the heavy mineral fraction of the most contaminated soil showed that the Cu is mainly present as Cu-(Fe) sulphides (chalcocite, chalcopyrite), metallic Cu, complex Cu-(Fe) oxides, and Cu-bearing sulphates. To determine the toxicity of these soils, we adopted a standardized OECD reproduction test using *Enchytraeus crypticus*. The number of reproduced enchytraeids correlated negatively with total Cu and Co concentrations ($r = -0.97$ and -0.94 at $p < 0.001$, respectively), and EDTA-extractable Cu ($r = -0.89$, $p < 0.001$); whereas no effects of the soil properties (pH, CEC, C_{org}) were observed. No reproduction was possible in soils with Cu levels >5000 mg/kg. Median effect concentration (EC50) was calculated for total Cu concentration, and corresponded to 351 mg/kg.

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1. Introduction

Soils in the vicinity of non-ferrous metal mining operations and smelters are often highly polluted by inorganic contaminants (metals, metalloids) resulting from deposited mining dust and smelter emissions (Ettler et al., 2005, 2011, 2012; Kříbek et al., 2010; Tembo et al., 2006). High levels of metal/metalloid contaminants and sulphur can induce toxicity for those organisms living in such soils, thus reducing the biological structure and function of the soil (Frouz et al., 2011; Salminen and Haimi, 1999; Tosza et al., 2010). Evaluation of soil toxicity is extremely complex, because it reflects not only the total content of pollutants, but also the pollutant bioavailability and interaction of pollutants with individual inorganic and organic soil compartments (Frouz et al., 2011). For this reason, biological testing of polluted soils is extremely useful for the overall assessment of soil toxicity and appropriately completes routine geochemical investigations.

Soil invertebrates play an important role in soil structuring, aeration, soil organic matter decomposition, and nutrient cycling

processes (Jänsch et al., 2005; Rocco et al., 2011; van Gestel et al., 2011). Enchytraeids (Oligochaeta) play this role in many soils worldwide (Jänsch et al., 2005) and are recommended as a test species within the OECD ecotoxicological testing programme (ISO, 2005; OECD, 2004), which among other factors enable studies to be compared worldwide. The enchytraeid reproduction test (ERT) was optimized for the species *Enchytraeus albidus* (see e.g. Amorim and Scott-Fordsmand, 2012), but can be performed using various species of the genus *Enchytraeus*; e.g. *Enchytraeus crypticus* is frequently used as an alternative model species to assess soil toxicity (Castro-Ferreira et al., 2012; Santorufu et al., 2012), having a shorter generation time and hence test period and different tolerance range to soil properties (pH, texture, organic matter) (Jänsch et al., 2005). As such, the latter species has also been tested in combination with various conditions e.g. density (Menezes-Oliveira et al., 2011) or combined with other soil organisms with so-called soil multi-species test systems (SMS) (Scott-Fordsmand et al., 2008). So far, compared to other invertebrate species (Conder et al., 2001; Godet et al., 2011; Spurgeon and Hopkin, 1996), *E. crypticus* has only marginally been used for the toxicity testing of soils from mining and smelting areas (e.g., Frouz et al., 2005).

In the majority of environmental studies in the African mining/smeltering districts, the risks for biota related to high metal/metalloid

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levels are routinely assessed using bulk concentration concepts (Křibek et al., 2010; Tembo et al., 2006) or by simple or sequential extractions mimicking “labile”, “plant-available”, or “bioaccessible” fractions (Ettler et al., 2011, 2012, 2014). To date, toxicity tests with organisms exposed to mining- and smelting-polluted soils from these areas are absent from the literature.

Therefore, the purpose of the present study, using *E. crypticus*, is to evaluate the toxicity of surface soils sampled around an active Cu smelter located in Mufulira, which according to previous screening studies by Křibek et al. (2010), is one of the contamination hotspots within the Zambian Copperbelt province. Moreover, the soil toxicity is correlated with total and “available” pollutant levels and other environmental factors.

2. Material and methods

2.1. Site description

With up to 675,000 t Cu mined annually, Zambia, is the world's 7th largest Cu producer (USGS, 2013); corresponding to approximately 4% of the world's total output of Cu and Co (Mobbs, 2010). The soil sampling for this study was based on a previous regional screening study (Křibek et al., 2010) in the vicinity of Cu-ore smelter located at Mufulira in the northern part of the Zambian Copperbelt (geographic coordinates are given in Table S1 in the Supplementary Data). The Mufulira copper smelter is the 27th largest in the world with regard to its smelting capacity, accounting for 230 kt per year (Schlesinger et al., 2011). The mining and smelting industries have been in operation for over 70 years in the Mufulira area and causing environmental contamination, which only started to be investigated in detail a few years ago (e.g. polluted soils, smelting and mining waste disposal) (Křibek et al., 2010; Vítková et al., 2010, 2011).

2.2. Soil sampling and analysis

Ten soil samples used in the present study were selected from a larger sample set presented by Ettler et al. (2014). Detailed locations of the soil profiles are given in Table S1 in the Supplementary Data,

and the major soil characteristics of the individual soil samples are presented in Table 1. The soils represent a typical pollution gradient in this area sampled at various distances from the smelter, following the predominant wind direction. The reference site was located at 24 km upwind from the smelter. In short, two profiles under different land use (grassland/forest area) were dug at each site. The most vulnerable topsoils (0–1 cm) were selected for all the sites. In addition, mainly for forested sites, where topsoils corresponded to organic horizons, also deeper mineral soil layers with lower contaminant levels were selected for comparison purposes in order to obtain a suitable concentration gradient. The soils were described according to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2006), and the sampled soils were air-dried and sieved through a 2-mm stainless steel sieve (Retsch, Germany). The <2 mm fraction was used for the measurement of pH, cation-exchange capacity (CEC), EDTA extractions, and a subsequent reproducibility test (see below). An aliquot of each sample was finely ground in an agate mortar (Fritsch Pulverisette 5, Germany) and used for the bulk chemical analyses.

The soil pH was measured by a Schott Handylab pH meter equipped with a Schott BlueLine 28 pH electrode (Schott, Germany) in a 1:5 (w/v) soil-deionised water suspension after 1-h agitation (Pansu and Gautheyrou, 2006). The cation exchange capacity (CEC) was determined as the sum of the basic cations; the Al was extracted with unbuffered 0.1 mol l⁻¹ BaCl₂ solution, and the extractable acidity determined (0.05 mol l⁻¹ NaOH titration) (Pansu and Gautheyrou, 2006). Total organic carbon (C_{org}) and total sulphur (S_{tot}) in the individual soil samples were determined using an Eltra CS 530 analyzer (Eltra, Germany). Metals (Co, Cu, Zn, and Pb) were determined in soil digests, after dissolution of the samples in mineral acids (HF–HClO₄–HNO₃), using inductively coupled plasma optical emission spectrometry (ICP-OES; ThermoScientific iCAP 6500, USA) (detailed procedure given in Ettler et al., 2013). The accuracy of the metal determinations in the soils was checked using parallel digestions and analyses of standard reference materials NIST 2710 (Montana II soil – highly elevated concentrations) and NIST 2711 (Montana soil – moderately elevated concentrations), and was found to be satisfactory (<9% relative standard deviation [RSD], Table S2 in the Supplementary Data).

Table 1
Description, location and properties of the studied soils^a.

Sample/code	Distance to smelter km	Soil type, description and depth			pH	C _{org} g/kg	S _{tot} mg/kg	CEC cmol +/kg
F1	3.6	Haplic Ferralsol Eutric, grassland area, 0–1 cm			5.46	51.6	810	5.89
E1	3.6	Haplic Ferralsol Eutric, forest area, 0–1 cm			5.39	89.2	744	15.1
E2	3.6	Haplic Ferralsol Eutric, forest area, 1–3 cm			6.30	9.8	229	4.07
E3	3.6	Haplic Ferralsol Eutric, forest area, 3–10 cm			6.70	11.4	217	5.34
A1	8	Haplic Plinthosol Eutric, grassland area, 0–1 cm			5.34	82.4	705	4.35
A4	8	Haplic Plinthosol Eutric, grassland area, 10–20 cm			5.15	10.5	105	3.81
O1	8	Haplic Ferralsol Xanthic, forest area, 0–1 cm			6.85	53.4	565	8.44
O3	8	Haplic Ferralsol Xanthic, forest area, 3–15 cm			5.78	20.6	105	3.47
G1 (ref. soil)	24	Stagnic Plinthosol Eutric, grassland area, 0–1 cm			6.55	28.5	229	8.98
H1 (ref. soil)	24	Haplic Ferralsol Dystric, forest area, 0–1 cm			5.10	19.9	103	3.75
KR (Danish ref. soil) ^b	–	Luvisol, Hygum agricultural area, Denmark, nd			5.80	29.0	nd	5.80
Sample/code	Cu mg/kg	Cu _{EDTA} mg/kg	Co mg/kg	Co _{EDTA} mg/kg	Pb mg/kg	Pb _{EDTA} mg/kg	Zn mg/kg	Zn _{EDTA} mg/kg
F1	8980 ± 60.5	3490 ± 80.5	45.8 ± 0.48	5.50 ± 0.14	41.6 ± 1.85	10.5 ± 0.17	63.2 ± 0.14	7.35 ± 0.08
E1	2830 ± 39.6	1570 ± 45.5	21.0 ± 0.06	7.68 ± 0.24	21.3 ± 0.14	8.77 ± 0.05	64.8 ± 0.52	19.7 ± 0.47
E2	1140 ± 7.50	533	20.0 ± 0.23	3.22	23.2 ± 2.10	4.03	55.0 ± 0.95	6.38
E3	1020 ± 5.52	482	19.9 ± 0.13	3.24	21.5 ± 0.22	3.95	51.4 ± 0.58	6.06
A1	5480 ± 22.0	1240 ± 21.0	25.2 ± 0.10	1.07 ± 0.09	39.1 ± 0.29	4.60 ± 0.07	83.3 ± 1.41	1.31 ± 0.03
A4	530 ± 448	30	14.2 ± 3.37	0.22	14.7 ± 6.62	1.92	49.0 ± 7.06	0.28
O1	3320 ± 58.2	1230 ± 21.5	23.5 ± 0.07	5.50 ± 0.08	17.9 ± 0.02	5.85 ± 0.06	50.8 ± 0.80	6.78 ± 0.08
O3	694 ± 24.4	335	5.41 ± 0.08	0.58	10.0 ± 0.03	2.41	28.5 ± 0.93	0.62
G1	37.4 ± 0.22	12.0 ± 0.16	2.70 ± 0.05	0.60 ± 0.02	2.5 (DL)	0.89 ± 0.01	25.1 ± 0.12	5.29 ± 0.03
H1	36.6 ± 5.76	15.4 ± 0.01	2.72 ± 0.02	0.83 ± 0.003	4.34	0.96 ± 0.01	15.5 ± 0.35	1.69 ± 0.12
KR	30	nd	nd	nd	nd	nd	nd	nd

nd = not determined.

^a Metal concentrations expressed as means ± standard deviations from duplicated analysis with some exceptions in EDTA extractions.

^b Data for this soil are taken from Amorim and Scott-Fordsmand (2012).

To determine the “labile” or “bioavailable” fraction of metals in the soils, a 0.05 mol/l EDTA extraction was performed on all soil samples according to the method described by Quevauviller (1998) (2 g soil in 20 ml 0.05 mol/l EDTA, shaken for 1 h, filtration to 0.45 µm). The concentrations of metals in the extracts were determined by quadrupole-based inductively coupled plasma mass spectrometry (ICP-MS; ThermoScientific XseriesII, USA). Certified reference material, BCR 483 (sewage sludge amended soil), was used to check the accuracy of the EDTA extractions, which were always <15% RSD with respect to the certified values (Table S2).

2.3. Soil mineralogy

The investigation of smelter-derived particles was carried out on the heavy mineral fraction, separated using 1,1,2,2-tetrabromomethane (density 2.96 g cm⁻³) from those soils with the highest pollution levels (F1 and A1, see Table 1). The material obtained was mounted into an epoxy resin and polished. The polished sections were studied using an optical microscope (Leica DM LP, Germany) and a Tescan VEGA (Tescan, Czech Republic) scanning electron microscope (SEM) operating at 15 kV with a beam current of 1.5 nA, and equipped with an Oxford Link X-Max 50 energy dispersion spectrometry (EDS) (Oxford Instruments, UK) calibrated against the SPI set of standards (SPI supplies, USA).

2.4. Toxicity test

The reproduction toxicity test was conducted in the National Environmental Research Institute (NERI), Aarhus University, Denmark, according to the standard guidelines (ISO, 2005; OECD, 2004), using *E. crypticus* as the test species. *E. crypticus* was cultured in the laboratory, kept at 20 °C in agar culture plates as a substrate and fed on dried oats. Adult organisms with visible clitellum and of approximately the same size were selected for the experiment. A mass of 20 g of soil was introduced in plastic containers and mixed with 20 mg of finely ground oats. Subsequently, approximately 2.5 ml of deionised water was added and 10 adult enchytraeids were introduced per container (total volume of water was 5 ml, corresponding to approximately 50% of the water holding capacity of the soils). The test was conducted in four replicates for all soil samples from Zambia ($n=10$), and in duplicate for the Danish reference soil. The test ran at 20 °C and a 12:8 hour photoperiod for 28 days. Food in the form of finely ground oats (10 mg) was added weekly. The water loss was replenished weekly by adding sufficient water to maintain the starting weight of the container. At the end of the test the enchytraeids were extracted by spreading the soil of each test into four 200 ml plastic beakers (7 cm in diameter), which were filled with deionised water, gently shaken and left for 24 h at 5 °C to stop reproduction and allow for soil sedimentation. Both adult and juvenile enchytraeids were gathered from each aliquot beaker and counted under a microscope within 48 h.

2.5. Data treatment

The data were evaluated using NCSS statistical software (NCSS, USA). Additionally, the data were tested for normality (Shapiro–Wilk test) and homogeneity of variance (Kruskal–Wallis test). Correlations between the individual parameters were expressed as Spearman rank correlation coefficients. Linear regression analysis was performed using NCSS scatter plots for individual parameters (transformed to log values), which was statistically highly correlated with the mean number of enchytraeids reproduced (confidence limit interval was expressed at $\alpha = 0.05$). The effective concentration causing a 50% reduction in the number of juveniles (EC50) was calculated using these linear regressions (Fig. 2).

Furthermore, a principle component analysis (PCA) was performed using Primer statistical software, version 6 (Clarke and Gorley, 2006), and was based on Euclidian distance measures for the environmental

Fig. 1. Scanning electron micrograph of particles from the heavy mineral fraction of the highly polluted soil F1 (in back-scattered electrons, BSE). Abbreviations: Cc – chalcocite (Cu₂S); Cpy – chalcopyrite (CuFeS₂); Ep – epidote (Ca₂Al₂(Fe,Al)(SiO₄)(Si₂O₇)O(OH)); Rt – rutile (TiO₂); Tnr – tenorite (CuO); Tur – tourmaline (mainly schorl: NaFe₃Al₆Si₆O₁₈(BO₃)₃(OH)₄); Zrn – zircon (ZrSiO₄).

variables (log transformed data), with the number of animals and Cu concentrations superimposed. A multidimensional scaling (MDS) statistical approach was also performed on the dataset, with similar results found (not reported in this paper).

3. Results

3.1. Bulk concentrations, EDTA extractability and solid speciation of metals

The bulk chemical analyses indicated that the total metal concentrations in the studied soils decreased with distance from the pollution source (smelter) as well as with depth (if applicable)

Fig. 2. Relationship between the numbers of reproduced *E. crypticus* and Cu concentration in soil as calculated by NCSS (confidence limit interval at $\alpha = 0.05$).

(Table 1). Concentrations of individual metals were in the ranges of 37–8980 mg Cu/kg, 3–46 mg Co/kg, <2.5–42 mg Pb/kg, and 16–83 mg Zn/kg; with the lowest values corresponding to topsoil from the reference sites located at 24 km upwind from the smelter.

The EDTA-extractable amounts of metals indicated that a portion of the contaminants is bound in the “labile” fractions of the soil: 12–3490 mg Cu/kg (6–55% of total Cu concentration), 0.2–7.7 mg Co/kg (2–36% of total Co), 0.9–10.5 mg Pb/kg (12–41% of total Pb) and 0.3–19.7 mg Zn/kg (1–30% of total Zn) (Table 1).

The SEM investigation of the heavy mineral fraction from the most contaminated topsoils revealed the presence of numerous rounded particles (smelter-derived) as well as angular grains (probably mining-derived) (Fig. 1). Primary metallic Cu and sulphides (predominant chalcocite, Cu_2S and chalcopyrite, CuFeS_2) were often surrounded with alteration rims composed of Cu-bearing Fe-oxides or sulphates (Fig. 1). Grains of binary Cu–Fe oxides occurred as rounded rim-like residual structures, indicating the high degree of their weathering (Fig. 1).

3.2. Reproduction test with *E. crypticus*

The results of the reproduction test with *E. crypticus* are summarized in Table 2. The number of juveniles obtained in the Danish reference soil (30 mg Cu/kg) was ca. 900, whereas significantly lower numbers were recorded for Zambian soils (e.g. by a factor of 0.44 for a similar Cu concentration). We are aware that this difference could partly be due to the difference in soil properties (Table 1). This observation could also be related to the relatively higher levels of naturally occurring labile Cu reported for these soil samples (EDTA-extractable Cu in the range of 32–42% of total Cu concentration) (Table 1). Reproduction was fully inhibited in those soils with the highest Cu concentrations (F1: 8980 mg Cu/kg; A1: 5480 mg Cu/kg). The correlation matrix between the number of enchytraeids and the soil's chemical and physicochemical parameters is reported in the Supplementary Data (Table S3). No statistically significant correlation was found with soil parameters (pH, CEC, C_{org}). The number of enchytraeids correlated significantly with the total Cu and Co concentrations ($r = -0.97$ and -0.94 at $p < 0.001$, respectively); however, correlation was also reported for other metals ($r = -0.84$ for Pb and $r = -0.82$ for Zn at $p < 0.05$). The number of enchytraeids also correlated with EDTA-extractable Cu ($r = -0.89$, $p < 0.001$) and EDTA-extractable Pb ($r = -0.88$, $p < 0.001$). As metallic contamination is closely related to sulphur in the vicinity of the Zambian smelters (Křibek et al., 2010), the number of reproduced enchytraeids also correlated with total sulphur ($r = -0.80$, $p < 0.05$). Regressions between the number of enchytraeids and selected parameters (total Cu, Co, and Cu_{EDTA}) were also performed (Fig. S1). Despite the fact that in multi-metal contaminated soils with correlations between individual metals, where the effects of the “cocktail” of contaminants could be observed (Godet et al., 2011), total Cu was assumed to be the most suitable predictor ($R^2 = 0.93$) (also see discussion). This regression line was

used for calculation of the median effect concentration (EC50) value, which corresponded to 351 mg Cu/kg. Based on other regressions with total Co and EDTA extractable Cu, additional EC50 values were 7.8 mg Co/kg and 99.5 mg Cu_{EDTA} /kg, respectively (Fig. S1).

4. Discussion

Copper and its toxicity to soil invertebrates have been widely investigated, due both to its common use in biocides/pesticides and its direct toxic action or food-chain accumulation in living organisms (Amorim and Scott-Fordsmand, 2012; Amorim et al., 2005; Criel et al., 2008; Menezes-Oliveira et al., 2011; Rocco et al., 2011; Scott-Fordsmand et al., 2008). Soil physicochemical properties can be used to predict toxicity using regression analysis (Criel et al., 2008; Frouz et al., 2005; Van Gestel et al., 2011). However, despite the similarity to the results obtained for *E. albidus* by Amorim et al. (2005), our data indicate that the observed range in the soil physicochemical parameters (pH, CEC, C_{org}) had no statistically significant correlation with the biological data. The results indicate that metal concentrations, i.e. total and/or their “labile” fraction (EDTA-extractable), are causing the decrease in the reproduction of enchytraeids. The strong correlation between the measured metals makes it difficult to identify the primary cause of toxicity. However, a comparison of the actual level with previously published metal toxicities to enchytraeids indicates that Cu is the primary cause of toxicity (see Fig. 3), with possible additional effects of other metals, e.g. Co in F1 or Zn in A1. It seems less likely that Pb is causing toxicity in the laboratory experiment, although long-term bioaccumulation may be an issue to native species in the area. Modelling the Cu toxicity to soil data (pH, CEC etc.) was attempted, using the CEC–EC50 relationship identified for soil organisms, but this gave no improvement of the correlation, possibly due to multiple toxicities.

The form and solid speciation of Cu in soils can significantly affect the population growth of the test species. In the majority of studies dealing with the Cu toxicity, soils are generally spiked with the soluble CuCl_2 salts (Amorim et al., 2005; Criel et al., 2008; Menezes-Oliveira et al., 2011; Rocco et al., 2011). Comparisons between the exposure of invertebrates to Cu salt and Cu (nano)particles have only recently started to be investigated (Amorim and Scott-Fordsmand, 2012; Amorim et al., 2012). In terms of energy allocations (as a proxy for body functions) among different energy reserves in *E. albidus*, no differences were observed between the two forms of Cu introduced into the soil (Amorim et al., 2012). However, significantly lower (2–8 fold) EC50 values were observed for the reproduction of *E. albidus* in soils with Cu nanoparticles compared to soils with Cu salts, corroborating with a nanoparticle-specific toxic effects (Amorim and Scott-Fordsmand, 2012). These authors hypothesize, that if Cu ions were released from nanoparticles exposed in the soil, they may have been bound to the soil constituents rather than remain as free ions in the soil solution. The observed toxic effects could also be related to the physical uptake of Cu nanoparticles by the organisms (Amorim and Scott-Fordsmand, 2012).

The mineralogical investigation of our highly polluted soils indicates that copper is the most important contaminant in the heavy mineral fraction, and generally forms particles $< 100 \mu\text{m}$ (Fig. 1). The mineralogy of these particles corresponds well to wind-dispersed slag dusts and smelter flue dusts collected in the Mufulira area, previously analysed by a combination of mineralogical and geochemical techniques (Křibek et al., 2010; Vítková et al., 2010, 2011). Primary sulphides from smelter dusts are supposed to be more stable than primary Cu sulphates, which are easily transformed upon leaching (Vítková et al., 2011); although alteration rims on sulphide particles from soils are indicating that even these compounds can be responsible for the release of Cu and associated contaminants due to strong chemical weathering. The final products of the weathering sequence of Cu–Fe sulphides are Cu–Fe oxides forming collapsed outer skeletons of initially rounded particles (Fig. 1; Ettler

Table 2

Number of juveniles from the reproduction test with *E. crypticus* in the respective polluted and reference soils (mean $\pm$ SD, $n = 4$).^a

Polluted soils			Reference soils		
Sample	Enchytraeids	Cu (mg/kg)	Sample	Enchytraeids	Cu (mg/kg)
F1	0 $\pm$ 0	8980	G1 (Zambia)	406 $\pm$ 70	37.4
A1	0 $\pm$ 0	5480	H1 (Zambia)	419 $\pm$ 42	36.6
O1	26 $\pm$ 16	3320	KR (Denmark) ^b	930 $\pm$ 33	30
E1	92 $\pm$ 60	2830			
E2	38 $\pm$ 36	1140			
E3	137 $\pm$ 118	1020			
O3	109 $\pm$ 90	694			
A4	149 $\pm$ 36	530			

^a Data ranged as a function of decreasing mean Cu concentration.

^b $n = 2$.

Fig. 3. PCA ordinations of environmental data superimposed with bubble plots of number of enchytraeids (a) and Cu concentration (b).

et al., 2013). The subsequent chemical weathering of these Cu-bearing particles in the soil is leading to the high release of Cu, representing the dominant contaminant in this soil ecosystem. Copper can subsequently be bound to the soil's adsorption sites and forms the "labile" Cu pool, which can be regarded as "potentially bioavailable", as is also confirmed by the high EDTA-extractable Cu concentrations in the most polluted soils (Table 1). Although the micrometre size of Cu-bearing particles probably does not enable their physical uptake by worms, as suggested by Amorim and Scott-Fordsmand (2012); for significantly smaller Cu nanoparticles, the possible toxic effects of uptake of discrete particles by soil organisms should also be considered.

5. Conclusions

A series of 10 soils from the vicinity of the Mufulira copper smelter (Zambian Copperbelt) with a Cu concentration gradient of 37–8980 mg/kg were investigated using the reproduction toxicity test with *E. crypticus* worm. The effective concentrations causing 50% reduction in the number of juveniles (EC50) corresponded to 351 mg Cu/kg. We hypothesize that the toxic effect of soils for this organism was primarily related to high levels of "labile" Cu (EDTA-extractable), which attained 55% of the total Cu concentration. Nevertheless, the toxic effects of Cu-bearing microparticles of aeolian origin (most likely slag dusts and smelter flue dusts), which were found in the most polluted soils, should also be considered and further investigated in soil invertebrates.

Acknowledgements

This study was supported by the Czech Science Foundation (GAČR 210/12/1413), and the Ministry of Education, Youth and Sports of the Czech Republic (MSM 0021620855). This study was carried out within the framework of the IGCP Project No. 594 ("Assessment of impact of mining and mineral processing on the environment and human health in Africa"). The toxicity test was carried out during the Erasmus stay of Ladislav Konečný at the Department of Geoscience and NERI (National Environmental Research Institute), Aarhus University, Denmark, which also funded this work. Laboratory and technical assistance were provided by a number of our colleagues: Marie Fayadová (chemical analyses and extractions), František Veselovský (heavy mineral fraction separation), and Martin Racek and Lucie Kovářová (SEM/EDS). Dr. Peter Lemkin is thanked for revision of the English within the manuscript. Reviews of two anonymous reviewers helped to improve the original version of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.gexplo.2013.10.004>.

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