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# Toxicity of Arsenate to the Compostworm *Eisenia fetida*, the Potworm *Enchytraeus albidus* and the Springtail *Folsomia candida*

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Elevated arsenic concentrations in terrestrial environments are associated with anthropogenic pollution through mining and smelting of arsenic ores or other metal ores with a high arsenic content, and application of arsenic-based pesticides used as for wood preservation (Tamaki and Frankenburger 1992). The dominant form of mineralized arsenic in oxic soils is arsenate, with small traces of arsenite and organic arsenicals (Cullen and Reimer 1989; Tamaki and Frankenburger 1992). Because of its widespread use and potential adverse effects on the environment, arsenic is receiving increased interest from various national and international regulatory organisations. However, the only toxicity data reported for soil invertebrates are a few studies on earthworms (Fischer and Koszorus 1992; Meharg et al. 1998; Langdon et al. 1999). The present study was therefore aimed at providing basic toxicity that are required to perform an environmental risk assessment of arsenic. The calculation of a predicted no effect concentration (PNEC) and a hazardous concentration for five percent of the species (HC5), which are used to derive soil quality criteria, rely on high quality chronic toxicity data, and therefore chronic toxicity tests were performed with the compostworm *Eisenia fetida*, the potworm *Enchytraeus albidus* and the springtail *Folsomia candida*, three invertebrates for which a standard test protocol is available.

## MATERIALS AND METHODS

The culture of *Enchytraeus albidus* Henle 1847 was kindly provided by J. Römbke. The culture substrate of *E. albidus* consists of artificial soil (OECD 1984) and animals were fed once a week with ground rolled oats (OECD 1999). The culture of *Folsomia candida* Willem 1902 was obtained from Aquasense B.V. (Amsterdam, The Netherlands). Animals are cultured on a substrate of plaster of Paris and pulverised chemical activated charcoal in a ratio of 8:1 (w:w) and granulated dry yeast is added as a food weekly (ISO 1999). The culture of *Eisenia fetida* Savigny 1826 was obtained from a commercial earthworm breeding farm. Animals are cultured in *Sphagnum* peat adjusted to pH 6-7 with $CaCO_3$ and are weekly fed with cow dung (OECD 1984). All cultures have been maintained in our laboratory for at least five years at 20°C.

Toxicity tests with *E. albidus* were carried out according to OECD Guideline 220

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(1999), chronic toxicity assays with *E. fetida* were performed as suggested by Van Gestel et al. (1989) and tests with the springtail *F. candida* were carried out according ISO (1999). In the chronic toxicity tests, 10 adult worms with a fully developed clitellum or 10 synchronised springtails of 10 to 12 days old were exposed per glass vessel which contained 20g wet weight of soil for *E. albidus*, 30g for *F. candida* and 750g for *E. fetida*. The artificial soil used as test substrate was composed as prescribed by OECD Guideline 207 (1984): 70% sand, 20% kaolin clay and 10% finely ground *Sphagnum* peat, adjusted to pH 6 with $CaCO_3$. Tests were carried out at a soil moisture content of 55 % of the water holding capacity. Chronic toxicity tests with *E. albidus* took six weeks to complete. Rolled oats were put on the soil surface weekly as a food source. After three weeks of exposure, the adults were removed and counted to evaluate acute toxicity and after another three weeks the juveniles were counted. To facilitate counting, the substrate was fixed with ethanol and a few drops of Bengal Red solution were added. The next day the substrate was washed through a 300µm sieve and the juveniles were retrieved and counted. The minimum number of 25 juveniles per vessel in the controls, as prescribed by the draft OECD Guideline 220 (1999), was always exceeded. The reproduction assay with *F. candida* took four weeks to complete. Granulated dry yeast was added weekly on the soil surface as food. At the end of the test, juveniles were counted after flotation. The number of juveniles in the controls always exceeded the prescribed minimum of 100 instars per vessel. In the chronic toxicity tests with *E. fetida*, adults were exposed for three weeks. At the start of the test, 2 % (dry weight) finely ground cow dung was supplied in a shallow depression in the test soil. At the end of the test, the number of cocoons was determined after washing the soil through a 1 mm sieve. During exposure, all test vessels were kept at 20 ± 1 °C and a light:dark cycle of 16:8 at 400-800 lux or constant illumination in the case of *E. fetida*. Soil moisture content was adjusted twice a week by replenishing weight loss with the appropriate amount of deionized water.

Arsenate was added as aqueous solutions ($Na_2HAsO_4.7H_2O$, Sigma, Steinheim, Germany, purity >98%). Arsenate was spiked in logarithmic series with four concentrations per order of magnitude. Nominal concentrations were 10, 18, 32, 56 and 100 mg As/kg dry wt, these concentrations were chosen on the basis of range-finding tests with the three organisms. For each concentration and the control, four replicates with ten organisms were tested.

The concentrations causing 50% effect (EC50s) with 95% confidence intervals were calculated using the probit method and the concentrations causing 50% mortality (LC50s) with 95% confidence intervals were calculated using the moving average method (Stephan 1977). No-observed effect concentrations (NOECs) were calculated by Kruskal-Wallis ANOVA followed by post-hoc multiple comparisons (Conover 1980).

Soils were digested in $HNO_3$/HCl/HF in a ratio of 4:1:1 (v:v:v) and soil arsenic concentrations were measured using ICP-AES. Nominal concentrations of the

spiked soils never differed more than 20 % from the measured concentrations. At the end of the exposures, pH (KCl) was measured with a pH meter (Consort, P407, Turnhout, Belgium) at a 1:2.5 soil:liquid ratio with 1M KCl (ISO 1994). Water holding capacity was determined by measuring the water content of the soil substrate after inundating the substrate for three hours and subsequently draining it for two hours (ISO 1996). The water content was determined by weighing the sample, drying it to constant mass at 105°C and re-weighing it.

## RESULTS AND DISCUSSION

For all test species, less than 10% of the control organisms died. The 21d LC50 for *E. albidus* was 90.2 (71.6-210) mg As/kg dry wt. No mortality occurred at the highest test concentration of 100 mg As/kg dry wt in the *E. fetida* tests and *F. candida* mortality was smaller than 10 % at this exposure concentration.

Reproduction of *F. candida* almost ceased at 56 mg As/kg dry wt (Fig. 1A) and the 28d EC50 was 13.1 (6.34-18.5) mg As/kg dry wt. At the lowest exposure concentration of 10 mg As/kg dry wt reproduction was significantly lower in comparison with the control and, therefore, no NOEC could be calculated. The 21d EC50, based on cocoon production, for *E. fetida* was 10.8 (4.73-14.3) mg As/kg dry wt and cocoon production ceased at 56 mg As/kg dry wt (Fig. 1B). The 42d EC50 (reproduction) for *E. albidus* was 22.9 (16.4-30.9) mg As/kg dry wt and no reproduction occurred at concentrations higher than 56 mg As/kg dry wt (Fig. 1C). The NOEC and LOEC for both *E. fetida* and *E. albidus* were 10 and 18 mg As/kg dry wt, respectively. These data indicate that arsenic toxicity in standard artificial soil (OECD 1984) occurs at concentrations about an order of magnitude lower than copper (Lock and Janssen 2001a), zinc (Lock and Janssen 2001b), cadmium (Lock and Janssen 2001c), nickel (Lock and Janssen (2001d) and chromium (Lock and Janssen 2001e) and more than two orders of magnitude lower than lead (Lock and Janssen 2001a), while mercury toxicity is similar (Lock and Janssen 2001f).

The toxicity of arsenic to terrestrial invertebrates is poorly documented: only three studies report on the toxicity of arsenic to earthworms while, to our knowledge, no data are available for other terrestrial invertebrates. Fischer and Koszorus (1992) found a 56d mortality around 50% for *E. fetida* exposed to 100 mg potassium arsenate/kg wet weight and the 56d cocoon production was lower than 50% when exposed to 50 mg potassium arsenate/kg wet weight in a substrate composed of peaty marshland soil mixed with horse manure. In a sandy loam soil collected below a beech-Scotch pine canopy, the 2d LC50 for *Lumbricus terrestris* was 400 arsenate/kg dry wt and the 8d LC50 was 100 mg arsenate/kg dry wt (Meharg et al. 1998). Furthermore, the toxicity of arsenate increased with depth down the soil profile, with the 4d LC50 decreasing from 300 to less than 100 mg As/kg dry wt, which was probably related with a decrease in organic matter content down the soil profile from 11.5 to 0.8 %. However, a population of *Lumbricus rubellus* was found on a mine spoil (Carrock Fell area) contaminated

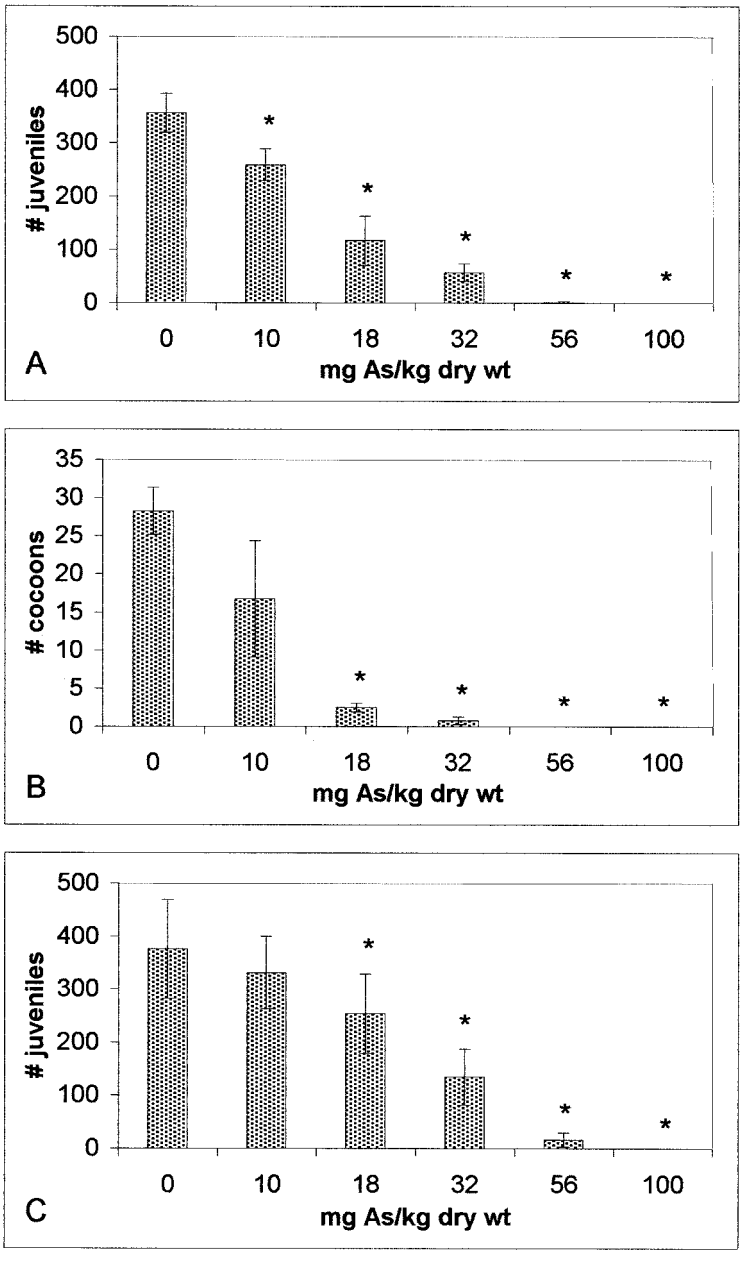


**Figure 1.** Reproduction (juvenile or cocoon production) of *Folsomia candida* (A), *Eisenia fetida* (B) and *Enchytraeus albidus* (C) exposed to arsenate for 28, 21 and 42 days, respectively (error bars = standard deviations; * = significantly different from the control with $p<0.05$).

with over 10000 mg As/kg dry wt whereas a population originating from an uncontaminated area was unable to survive in this contaminated soil (Langdon et al. 1999). This indicates that earthworms are, to a certain degree, able to acclimate or adapt to elevated arsenic concentrations. However, it would be totally unjustified to view the evolution of resistance as a justification for the release of arsenic into the environment.

Differences in soil properties between our study and the above mentioned literature data make it difficult to compare the test results as it has been shown that soil characteristics can have a great influence on the bioavailability and toxicity of metals (Lock et al. 2000; Lock and Janssen 2001a): toxicity data for Zn, Cd, Cu and Pb varied over more than two orders of magnitude depending on the used soil. Cation exchange capacity and pH were the most important soil parameters determining metal ecotoxicity and the 14d LC50 for *E. albidus* could be well predicted on the basis of these parameters. However, too few data are currently available for arsenic to evaluate the importance of soil parameters such as pH and cation exchange capacity on the ecotoxicity of arsenic. Furthermore, all toxicity tests with arsenic were carried out with freshly spiked soils and therefore the effect of aging is not taken into account. The importance of soil parameters on arsenic toxicity and the effect of aging need to be addressed urgently to allow the development of ecologically relevant risk assessment procedures for arsenic contaminated soils.

## REFERENCES


Conover WJ (1980) Practical non-parametric statistics. J. Wiley & Sons, New York.

Cullen WR, Reimer KJ (1989) Arsenic speciation in the environment. Chem Rev 89:713-764.

Fischer E, Koszorus L (1992) Sublethal effects, accumulation capacities and elimination rates of As, Hg, and Se in the manure worm, *Eisenia fetida* (Oligochaeta, Lumbricidae). Pedobiologia 36:172-178.

ISO (1994) Soil Quality - Determination of pH, No. 10390. International Organisation for Standardisation, Geneve.

ISO (1996) Soil Quality - Effects of pollutants on earthworms (*Eisenia fetida*). Part 2: Determination of effects on reproduction, No. 11268-2. International Organisation for Standardisation, Geneve.

ISO (1999) Soil Quality - Effects of soil pollutants on Collembola (*Folsomia candida*): method for determination of effects on reproduction. No. 11267. International Organisation for Standardisation, Geneve.

Langdon CJ, Piearce TG, Black S, Semple KT (1999) Resistance to arsenic-toxicity in a population of the earthworm *Lumbricus rubellus*. Soil Biol Biochem 31:1963-1967.

Lock K, Janssen CR, De Coen WM (2000) Multivariate test designs to assess the influence of zinc and cadmium bioavailability in soils on the toxicity to

*Enchytraeus albidus*. Environ Toxicol Chem 19:2666-2671.
Lock K, Janssen CR (2001a) Test designs to assess the influence of soil characteristics on the toxicity of copper and lead to the oligochaete *Enchytraeus albidus*. Ecotoxicology 10:137-144.
Lock K, Janssen CR (2001b) Modelling zinc toxicity for terrestrial invertebrates. Environ Toxicol Chem 20:1901-1908.
Lock K, Janssen CR (2001c) Cadmium toxicity for terrestrial invertebrates: taking soil parameters affecting bioavailability into account. Ecotoxicology 10:315-322.
Lock K, Janssen CR (2001d) Ecotoxicity of nickel to *Eisenia fetida*, *Enchytraeus albidus* and *Folsomia candida*. Chemosphere:in press.
Lock K, Janssen CR (2001e) Ecotoxicity of chromium(III) to *Eisenia fetida*, *Enchytraeus albidus* and *Folsomia candida*. Ecotoxicol Environ Saf:submitted.
Lock K, Janssen CR (2001f) Ecotoxicity of mercury to *Eisenia fetida*, *Enchytraeus albidus* and *Folsomia candida*. Biol Fert Soils 34:219-221.
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.
OECD (1984) Earthworm, acute toxicity tests. OECD-guideline for testing chemicals 207, Organisation for Economic Cooperation and Development, Paris.
OECD (1999) Enchytraeid reproduction test. Draft OECD-guideline for testing chemicals 220, Organisation for Economic Cooperation and Development, Paris.
Stephan C (1977) Methods for calculating an LC50. ASTM (American Society for Testing and Materials), Special Technical Publication 34:65-84.
Tamaki S, Frankenburger WT (1992) Environmental biochemistry of arsenic. Rev Environ Contam Toxicol 124:79-110.
Van Gestel CAM, van Dis WA, van Breemen EM, Sparenburg PM (1989) Development of a standardised reproduction toxicity test with the earthworm species *Eisenia andrei* using copper, pentachlorophenol, and 2,4-dichloroaniline. Ecotoxicol Environ Saf 18:305-312.