

## Effects of microelements in calcareous loamy chernozem soil on *Enchytraeus albidus* under laboratory conditions

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**Abstract.** Effects of six microelements (Cr, Cu, Hg, Pb, Zn and Se) were tested under laboratory conditions on the mortality and reproductive success of *Enchytraeus albidus*. LOEC<sub>mortality</sub> was 7 mg/kg of available Se and LOEC<sub>reproduction</sub> 2 mg/kg of available Se. No mortality effect was found in the case of available Cr, Cu, Hg, Pb and Zn at rates of 1.5, 131, 17, 167, and 124 mg/kg, respectively, but all these elements in the given concentration decreased reproduction. Available Zn decreased reproduction already at a rate of 47 mg/kg.

**Key words:** *Enchytraeus albidus*, microelements, toxicity.

### INTRODUCTION

*Enchytraeus albidus* as an ecotoxicological tool is relatively new in soil toxicological testing (Römbke 2003). Consequently, few data are available about the microelement toxicity on this species. In the study, chronic toxicity tests were carried out with field aged calcareous loamy chernozem soils under laboratory conditions. The effects of six microelements (Cr, Cu, Hg, Pb, Zn, and Se) aged for 7 years on the field were tested on the mortality and reproductive success of *E. albidus* in order to evaluate whether the relatively high doses of the applied heavy metals are still effective on *E. albidus* after 7 years.

### MATERIALS AND METHODS

Soil samples originated from the experimental farm of the Research Institute for Soil Science and Agricultural Chemistry at Nagyhorcsök (Hungary). Plots

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**Table 1.** Microelement concentrations of calcareous loamy chernozem soil in Nagyhörösök, Hungary. Total concentrations were measured in soils sampled in 1994. Available concentrations were measured in soils sampled in 1998. High and low pollution refers to the relative microelement concentration of the soils. For available concentrations of Se see Fig. 1

| Elements | High pollution soil |                        | Low pollution soil |                        |
|----------|---------------------|------------------------|--------------------|------------------------|
|          | Total <sup>a</sup>  | Available <sup>b</sup> | Total <sup>a</sup> | Available <sup>b</sup> |
| Cr       | 121                 | 1.5                    | 64                 | 0.9                    |
| Cu       | 230                 | 131                    | 85                 | 51                     |
| Hg       | 157                 | 17                     | 67                 | 2.2                    |
| Pb       | 264                 | 167                    | 142                | 87                     |
| Zn       | 274                 | 124                    | 118                | 47                     |

<sup>a</sup> ccHNO<sub>3</sub> + ccH<sub>2</sub>O<sub>2</sub> soluble microelements (Hungarian standard MSZ: 21470-50).

<sup>b</sup> NH<sub>4</sub> acetate + EDTA soluble microelements (Lakanen & Erviö 1971).

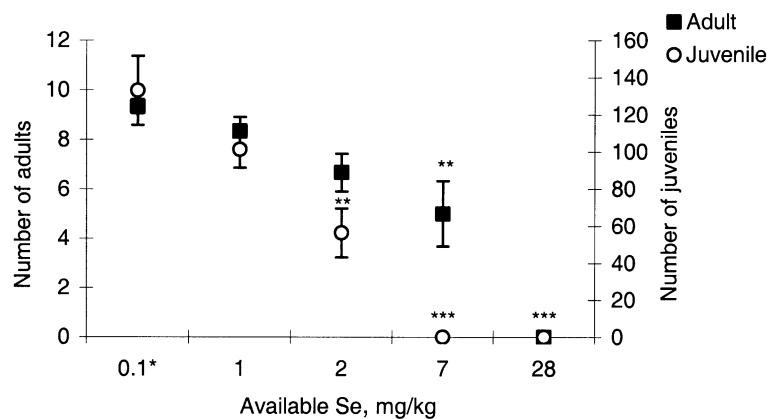
received 10, 30, 90, or 270 mg/kg Se nominal load and 90 or 270 mg/kg Cr, Cu, Hg, Pb, and Zn nominal load as single metal application ploughed in the upper 20 cm of the soil in 1991. Thereafter normal agricultural activity was practised on the 21 m<sup>2</sup> treated and control plots each year. Soils were sampled and transferred to the laboratory from these plots in 1998. The soil of the experimental plots is a calcareous loamy chernozem with a medium to deep humus layer formed on loess. Exchangeable cations comprised 80% Ca, 16% Mg, 3% K, and 1% Na; pH<sub>(KCl)</sub> was 7.4. For further description of the site and experiment, see Nagy (1999). Total (measured in 1994) and available (NH<sub>4</sub> acetate + EDTA soluble) in 1998 concentration values for all the studied heavy metals are presented in Table 1. The tests were carried out according to OECD (2003). After drying and sieving on a 2 × 2 mm mesh soil samples (20 g dry weight, 55% water capacity) were put into 100 mL culture boxes, 0.6 g grained oat flakes were mixed into the soil samples, and 10 egg-containing adult *E. albidus* were placed on them from our standard laboratory cultures. The boxes were sealed with parafilm and randomly placed into a thermostat. Animals were kept at 18°C (±0.8°C) temperature and 75% (±5%) humidity. The culture boxes were opened twice a week and the health status of the animals as well as the humidity of the soil was checked. Once a week grained oat flakes were fed to the animals. Five replicates per treatment were used, and the experiments lasted 6 weeks. Data of the Se experiment were analysed by ANOVA and thereafter LSD. Results gained on treated soils were compared to the control by two-sided *t*-test in all other cases.

## RESULTS AND DISCUSSION

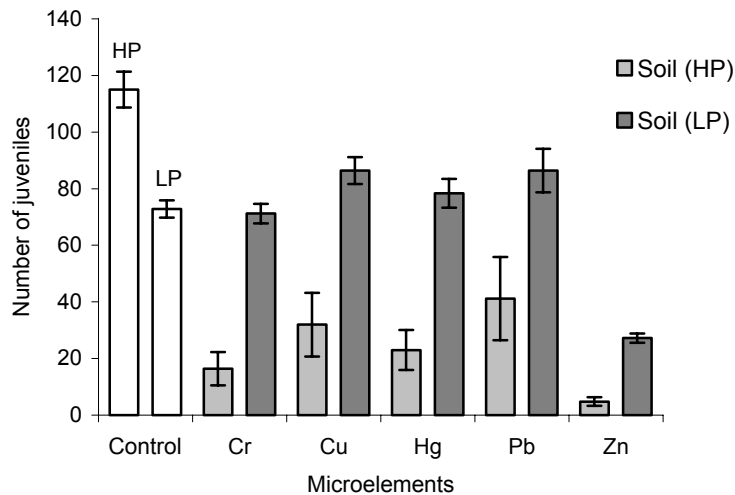
All six elements had certain effects on the tested parameters 7 years after application. A significant mortality effect was found in the case of available Se at a rate of 7 (total 81) mg/kg (LOEC<sub>mortality</sub>) while 2 mg/kg available (total 29) Se

( $LOEC_{\text{reproduction}}$ ) decreased reproduction (Fig. 1). Both figures are higher than the current threshold limit (1 mg/kg total Se) in Hungary. No comparable data are available in the literature regarding Se effect on *E. albidus*.

No mortality effect was found in the case of available Cr, Cu, Hg, Pb, and Zn at rates of 1.5, 131, 17, 167, and 124 mg/kg, respectively, but all these elements in the given concentrations decreased reproduction. Lower available concentrations (Table 1) affected neither mortality nor reproduction except in the case of 47 mg/kg available Zn, which decreased reproduction (Fig. 2). In this experiment Cr proved



**Fig. 1.** Average abundance of *E. albidus* adults and juveniles per pot with the increasing Se concentrations. Bars represent  $\pm$ SD. Significance levels: \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ; \* concentration in the control soil.



**Fig. 2.** Average number of juveniles in different treatments (results of two subsequent experiments). Bars represent  $\pm$ SD. Available concentrations in high pollution (HP) soils: 1.5 mg/kg Cr, 131 mg/kg Cu, 17 mg/kg Hg, 167 mg/kg Pb, and 124 mg/kg Zn; and in low pollution (LP) soils: 0.9 mg/kg Cr, 51 mg/kg Cu, 2.2 mg/kg Hg, 87 mg/kg Pb, and 47 mg/kg Zn. Significance level:  $p < 0.001$ .

to be notably more toxic for *E. albidus* than found by Lock & Janssen (2001a), who conducted their experiments on standard OECD soil. The reason of the contradictory finding is not clear in spite of the fact that originally the more toxic Cr(VI) was applied in this experiment, but 7 years on the field was enough to transform Cr(VI) to Cr(III). All other data are of the same order of magnitude as presented by Lock & Janssen (2001b, 2002).

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## **Mikroelementide toime liigile *Enchytraeus albidus* saviliivlõimisega karbonaatses mustmullas, uuritud laborikatses**

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Uuriti kuue mikroelemendi (Cr, Cu, Hg, Pb, Zn ja Se) mõju hariliku valge-liimuka (*Enchytraeus albidus*) suremusele ja sigimisedukusele laboritingimustes. Seeleni puhul suurendas suremust elemendi kättesaadav kontsentratsioon mullas vähemalt 7 mg/kg ja sigimist pärssis juba 2 mg/kg. Teised elemendid (Cr, Cu, Hg, Pb ja Zn), kontsentratsioon vastavalt 1,5, 131, 17, 167 ja 124 mg/kg, suremust ei mõjutanud, küll aga pärssisid sigimist. Zn tegi seda ka kontsentratsioonil 47 mg/kg.