Ecotoxicology (2007) 16:379–384
DOI 10.1007/s10646-007-0140-6

Toxicity of selenate and selenite to the potworm *Enchytraeus albidus* (Annelida: Enchytraeidae): a laboratory test

Zoltán Somogyi · István Kiss · Imre Kádár · Gábor Bakonyi

Accepted: 21 February 2007 / Published online: 24 March 2007

Abstract Little information is available about the toxicity of inorganic selenium forms in soil animals. Therefore, the effects of selenate and selenite on the mortality and reproduction of *Enchytraeus albidus* were examined in standard laboratory tests with chronic exposure. Total and available amount of selenate and selenite were tested in a calcareous loamy chernozem soil. The LC_{50} of selenate was 5.69 (2.7–8.12) mg kg^{-1} dry wt. for total Se and 4.74 (2.14–6.98) mg kg^{-1} dry wt. for available Se. Selenite LC_{50} was as high as 22.5 (19.6–25.7) mg kg^{-1} dry wt. for total Se and 8.10 (6.8–9.6) mg kg^{-1} dry wt. for available Se. The EC_{50} of selenate was 0.41 (0.35–0.48) mg kg^{-1} dry wt. for total Se and 0.28 (0.24–0.34) mg kg^{-1} dry wt. for available Se. Selenite EC_{50} was as high as 7.3 (6.2–8.5) mg kg^{-1} dry wt. for total Se and 2.46 (2.05–2.91) mg kg^{-1} dry wt. for available Se. The response in reproduction was more sensitive to Se toxicity than the response in mortality. Selenate proved to be more toxic than selenite. Available data show that *E. albidus* may function as a biological indicator for some inorganic selenium forms in the soil.

Keywords Selenite · Selenate · Potworm · *Enchytraeus albidus* · Toxicity

Z. Somogyi · I. Kiss · G. Bakonyi (✉)
Department of Zoology and Ecology, Szent István University, Páter K. u. 1, 2100 Gödöllő, Hungary
e-mail: bakonyi.gabor@mkk.szie.hu

I. Kádár
Research Institute for Soil Science and Agricultural Chemistry, Hungarian Academy of Sciences, Herman O. u. 15, 1025 Budapest, Hungary

Introduction

Selenium concentration is highly variable ranging from nearly zero up to 1,250 mg kg^{-1} in some soils in Ireland (Oldfield 1999). The total ($ccHNO_3$ + ccH_2O_2 soluble) concentrations are between 0.03 and 2.0 mg kg^{-1} in Hungary, but as high a concentration as 5.0 mg kg^{-1} occurs in some regions, for instance, in the Bükk Mountains (Kádár 1998). The assessment threshold for total selenium in soil is 3 mg kg^{-1} in the European Union (Kabata-Pendias and Adriano 1995). Selenium is a potentially toxic element to soil animals because of its biomagnification ability in food chains (Wu 2004). The ecological mechanisms of toxicity on soil animals may be rather complicated because of the several forms of selenium in soils and different biotransformation and accumulation processes (Jensen et al. 2005).

Little is known about the ways selenium affects soil animals. The earthworm *Eisenia fetida* was studied in laboratory conditions (Fischer and Koszorus 1992). A wide difference was found between the lethal and sublethal concentrations of selenite. The maximum Se concentration in earthworm body was as high as 337.6 mg kg^{-1} which may be extremely hazardous to earthworm predators (Fischer and Koszorus 1992). Jensen et al. (2005) studied the effects of four selenium forms on *Megaselia scalaris* larvae (Diptera, Phoridae). Organic forms of selenium proved to be more detrimental to larval development than inorganic forms. Selenate was, in general, a more toxic form of selenium than selenite. All results published on the effect of selenite or selenate on *E. fetida* were gained by environmentally relevant chronic laboratory tests.

The potworm *E. albidus* is an obvious target species to selenium toxicity since this species feeds on both mineral and organic components of the soil. Therefore, both the inorganic and organic forms of selenium may affect this species. There are no available data about the toxicity of selenium on *E. albidus* except those of Somogyi et al. (2005), who worked with a similar method and soil type as in the present study. In their study, soil samples with different Se concentrations were collected from a field experiment (Kádár 1994) after 7 years of 10, 30, 90 or 270 mg kg^{-1} Se (in a form of $Na_2O_3Se \cdot 5H_2O$) application. No further spiking was applied, but the field aged soils were tested immediately after sampling. It was found that the lowest effect concentration was 7 and 2 mg kg^{-1} dry wt. available Se for mortality and reproduction, respectively (Somogyi et al. 2005).

With regard to the little information published about the toxicity of selenium to soil animals, the effects of selenium on the mortality and reproduction of the potworm *E. albidus* was tested in order to study this species as an indicator of selenium pollution. The study aimed at revealing the degree of differences in the effects of selenate and selenite on the above parameters by spiking a field soil with the two selenium species.

Material and methods

Soil samples were collected from the control plots of the experimental field of the Research Institute for Soil Science and Agricultural Chemistry (RISSAC) of the Hungarian Academy of Sciences at Nagyhörcsök, Hungary (UTM-code: CT 00). The soil type of the experimental plots is a calcareous loamy chernozem with medium-to-deep humus layer formed on loess. Exchangeable cations comprise of 80% Ca, 16% Mg, 3% K, 1% Na. S: 40 meq 100 g^{-1}, $pH_{(KCl)}$ 7.1, humus 3.1%. For further description of the site, see Kádár (1994), Nagy (1999) and Nagy et al. (2004). The soil was spiked by Na-selenite ($Na_2O_3Se \cdot 5H_2O$, Merck, No. 106607) and Na-selenate (Na_2O_4Se, Sigma-Aldrich, No. 71948) in six different concentrations in the laboratory. Soils were differently spiked for tests with juveniles and adults (Tables 1 and 2). For the mortality tests, a wider range of the concentrations was used than for the reproduction tests. Soils were sampled from each concentration 24–48 h after spiking and stored at 4 °C until Se analyses. Se concentration was determined in the ICP Laboratory of the RISSAC. Total ($ccHNO_3 + ccH_2O_2$ soluble) concentrations were measured using the Hungarian standard MSZ: 21470-50, while available (NH_4-acetate + EDTA soluble) concentrations after Lakanen and Erviö (1971). The detection limit was 0.6 and 0.12 mg kg^{-1} for total and available concentrations, respectively.

Chronic toxicity tests used in this experiment were based on the OECD guideline No. 220 (OECD 2003). The animals originated from the *E. albidus* stock cultures of the Department of Zoology and Ecology, Szent István University (Gödöllő, Hungary). Containers of 150 ml were filled with 20 g oven dry soil. Na-selenite and Na-selenate solutions with different concentrations were prepared, and the soil was watered to 55% WHC with these solutions. Ten adult worms with fully developed clitellum and visible eggs were put in each test pot at the start of the experiment. Containers were covered by their own cups and randomly placed into a thermostat. The tests were performed at 18 ± 0.8 °C and 80% RH in total darkness. The animals were fed with pulverised oat-flake once a week. The health status of the animals, as well as the soil water content was checked once a week. Five replicates were used per concentration. Containers were destructively sampled after 6 weeks. Mortality and reproduction were measured as end points. Calculations of LC_{50} and LC_{10} (mortality), EC_{50}

Table 1 Total and available concentrations of Se in Na-selenate and Na-selenite spiked soils used for the mortality tests

Na-selenate		Na-selenite	
Total	Available	Total	Available
0.46[a]	0.17	0.77	0.19
4.41	3.57	6.21	1.83
8.76	7.66	12.8	3.90
17.00	15.80	24.8	9.28
29.45	29.10	42.6	17.55
51.65	50.70	76.6	35.80
94.48	90.80	132.0	69.10

Values are expressed as mg kg^{-1} dry wt. Control soil data are in the first row

Total: $ccHNO_3 + ccH_2O_2$ soluble, available: NH_4-acetate + EDTA soluble

[a] Below or about the detection limit

Table 2 Total and available concentrations of Se in Na-selenite and Na-selenate spiked soils used for the reproduction tests

Na-selenate		Na-selenite	
Total	Available	Total	Available
0.49[a]	0.12[a]	0.23[a]	0.11[a]
0.38[a]	0.23	1.20	0.37
0.59[a]	0.42	2.35	0.80
1.09	0.82	5.08	1.62
2.11	1.86	9.975	3.51
4.79	4.79	20.55	8.20
10.15	10.01	43.95	19.95

Values are expressed as mg kg^{-1} dry wt. Control soil data are in the first row

Total: $ccHNO_3 + ccH_2O_2$ soluble, available: NH_4-acetate + EDTA soluble

[a] Below or about the detection limit

and EC_{10} (reproduction), as well as NOEC were made by the ToxRat (2003) statistical software. The homogeinity of the data was tested by Cochran's test. In the case of homogeneous variances, *t* test and Williams multiple sequential *t* test (NOEC) were calculated. Data with inhomogeneous variances were analysed by Welch *t* test or by Welch *t* with Bonferroni adjustment (NOEC). Probit analysis was based on linear maximum likelihood regression. The slope of the dose–response regression curve was used as an estimator of tolerance range of the selenate or selenite toxicity. In the case of a normal distribution of the data, if the slope value will show to be higher, then the tolerance range narrower for a given factor.

Results

There were essential differences in total and available concentrations of selenate and selenite (Tables 1 and 2). Total amount of selenate applied remained more or less in available form in the soil. However, a significant amount of selenite was absorbed by soil components. The ratio of the available Se enhanced continuously till the highest concentrations applied (Table 3). This trend was observed both for selenate and selenite. Seventy-five to hundred percent of selenate remained available depending on the concentration applied. This range was 25–52% in the case of selenite.

Control mortality was less than 15% in each experiment. Selenate was more toxic than selenite both in the mortality and reproduction test (Table 4 and 5). The LC_{50} of the selenate (5.69 mg kg^{-1} dry wt. for total Se and 4.74 mg kg^{-1} dry wt. for available Se in average) proved to be remarkably lower than that of selenite (22.5 mg kg^{-1} dry wt. for total Se and 8.10 mg kg^{-1} dry wt. for available Se in average). Differences in NOEC were lower. A lower NOEC of selenate was found for the total concentration and a higher one for the available concentration (Table 4).

Table 3 Ratio of soil total and available selenite and selenate concentrations (%)

Selenate		Selenite	
AM/TM (%)	AR/TR (%)	AM/TM (%)	AR/TR (%)
37.0[a]	24.5[a]	24.7	47.8[a]
81.0	60.5[a]	29.5	30.8
87.4	71.2[a]	30.5	34.0
92.9	75.2	37.4	31.9
98.8	88.2	41.2	35.2
98.2	100.0	46.7	39.9
96.1	98.6	52.4	45.4

AM available concentration in mortality test; *TM* total concentration in mortality test; *AR* available concentration in reproduction test; *TR* total concentration in reproduction test. Control soil data are in the first row

Basic data as seen in Tables 1 and 2

[a] Original data are below or about the detection limit

The response in reproduction was more sensitive to Se toxicity than the response in mortality (Table 5). The EC_{50} of selenate was about one order of magnitude lower (0.41 mg kg^{-1} dry wt. for total Se and 0.28 mg kg^{-1} dry wt. for available Se in average) than that of selenite (7.3 mg kg^{-1} dry wt. for total Se and 2.46 mg kg^{-1} dry wt. for available Se in average). NOEC for total and available Se concentrations was higher after selenite compared to selenate treatment (Table 5).

Adult mortality on the selenate spiked soil was significantly higher than that of the control. This was apparent already at the lowest concentration tested (4.41 mg kg^{-1} dry wt. total and 3.57 mg kg^{-1} dry wt. available concentration) (Fig. 1). Similar effect was found in the reproduction test, where only about two-thirds of juveniles were produced compared to the control due to the lowest concentration tested (0.38 mg kg^{-1} dry wt. total and 0.23 mg kg^{-1} dry wt. available concentration mg kg^{-1} dry wt.) (Fig. 2).

Selenite enhanced adult mortality at a concentration of 12.8 mg kg^{-1} dry wt. total and 3.9 mg kg^{-1} dry wt. available concentration) (Fig. 3). Both values are higher than those of selenate. Fewer juveniles were produced compared to the control if the soil had a concentration of 5.08 mg kg^{-1} dry wt. total and 1.62 mg kg^{-1} dry wt. available selenite (Fig. 4).

Tolerance of *E. albidus* for selenate and selenite was estimated by the slope of the lines fitted to the data with probit analysis (Table 6). It was found that reproduction is more sensitive to concentration changes than mortality. The reaction of mortality to concentration changes is more or less the same both for selenate and selenite. However, the response in reproduction is more sensitive to changes of selenate concentration than to changes in selenite concentration.

Discussion

Contrary to the above-ground herbivores which predominantly take up organic forms of selenium from the plants (Trumble et al. 1998), in the food of the potworm the inorganic forms of selenium may also be present (Didden and Römbke 2001; Römbke 2003). Selenium toxicity data based on total soil concentrations show that this microelement is more harmful for *E. albidus* than heavy metals like cadmium (Lock and Janssen 2001a), mercury (Lock and Janssen 2001b), chromium (III) (Lock and Janssen 2002a), zinc, cadmium, copper and lead (Lock and Janssen

Table 4 Lethal and no-effect concentrations of Se in Na-selenite and Na-selenate spiked soils used for the mortality tests

	Na-selenate		Na-selenite	
	Total	Available	Total	Available
LC_{10}	0.97 (0.06–2.25)	0.67 (0.03–1.68)	7.3 (5.07–6.21)	2.05 (1.31–2.77)
LC_{50}	5.69 (2.7–8.12)	4.74 (2.14–6.98)	22.5 (19.6–25.7)	8.10 (6.8–9.6)
NOEC	4.41	3.57	6.21	1.83

Values are expressed as mg kg^{-1} dry wt. 95% confidence limits are in parentheses. Total: $ccHNO_3$ + ccH_2O_2 soluble, available: NH_4-acetate + EDTA soluble

Table 5 Lethal and no-effect concentrations of Se in Na-selenite and Na-selenate spiked soils used for the reproduction tests

	Na-selenate		Na-selenite	
	Total	Available	Total	Available
EC_{10}	0.29 (0.12–0.34)	0.19 (0.07–0.23)	2.8 (1.61–3.74)	0.82 (0.46–1.12)
EC_{50}	0.41 (0.35–0.48)	0.28 (0.24–0.34)	7.3 (6.2–8.5)	2.46 (2.05–2.91)
NOEC	0.38	0.23	2.35	0.8

Values are expressed as mg kg^{-1} dry wt. 95% confidence limits are in parentheses. Total: $ccHNO_3$ + ccH_2O_2 soluble, available: NH_4-acetate + EDTA soluble

2002b). Comparing the toxicity of selenate and selenite (this study) and heavy metals (data presented in the above-mentioned papers) on *E. albidus*, the difference is about one order of magnitude in most cases. However, the toxicity of pore water zinc on the mortality and reproduction of *E. albidus* fell in the some order of magnitude Lock and Janssen (2003) as that of selenate and selenite in this experiment.

In spite of the fact that Fischer and Koszorus (1992) did not calculate LC_{50} and/or EC_{50} values in their study, it is clear from their paper (Figs. 1 and 3) that both the mortality and the reproduction of *E. fetida* were less influenced by selenite than the same parameters of *E. albidus* in this experiment. The difference is about at least one order of magnitude. LC_{50} values for the soil inhabiting larvae of the small fly *M. scalaris* were 258 mg kg^{-1} wet weight and 392 mg kg^{-1} wet weight for selenate and selenite, respectively (Jensen et al. 2005). These are substantially higher values compared to the ones we found for *E. albidus*. Due to the lack of sufficient data, it is not possible to position *E. albidus* in a wider range of selenium toxicity of soil animals. However, available data show that this is a relatively sensitive species to inorganic selenium forms.

Somogyi et al. (2005) found that sodium-selenite application is still highly toxic to *E. albidus* 7 years after application. Their data are in the same order of magnitude as presented in this study in spite of the fact that in our experiments freshly spiked soil was used. This means that selenium can keep a high level of toxicity on the field for a long time.

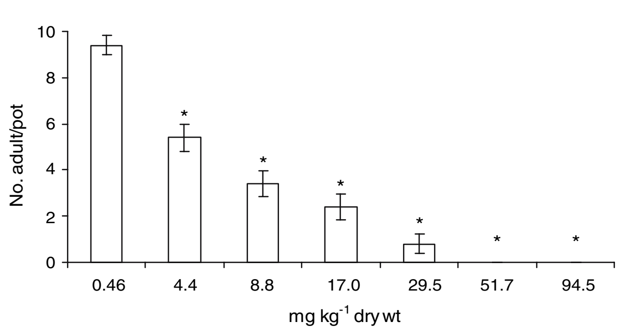

Fig. 1 Mortality of *Enchytraeus albidus* exposed to different concentrations of Na-selenate for 42 days. *Significant difference to control with $p < 0.05$. Data of the total concentrations are shown in *x*-axis

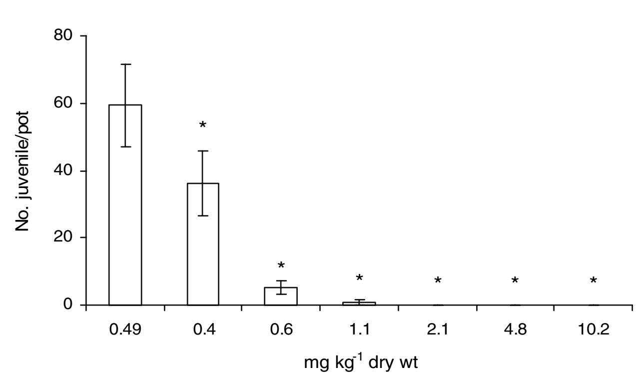

Fig. 2 Reproduction of *E. albidus* exposed to different concentrations of Na-selenate for 42 days. *Significant difference to control with $p < 0.05$. Data of the total concentrations are shown in *x*-axis

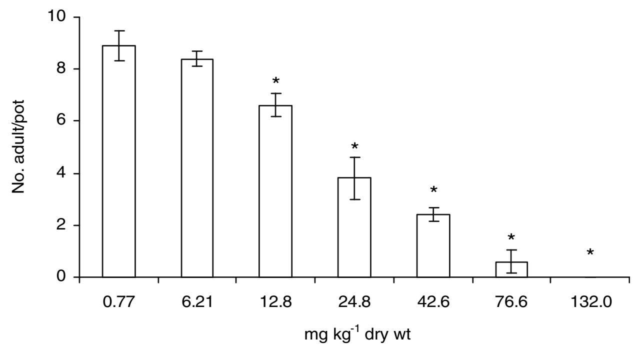

Fig. 3 Mortality of *E. albidus* exposed to different concentrations of Na-selenite for 42 days. *Significant difference to control with $p < 0.05$. Data of the total concentrations are shown in *x*-axis

There are great differences between species concerning how toxic substances may affect their life history parameters. Crommentuijn et al. (1955) studied the effect of cadmium exposure on four different soil animal species. It was found that on the basis of sublethal effects, *Platynothrus peltifer* (Oribatida) is the most sensitive species while on the basis of lethal effects *Orchesella cincta* (Collembola) proved to be the most sensitive one. In the case of *E. albidus*, tolerance of reproduction for selenate and selenite is narrower than that of mortality. Therefore, the reproduction test of *E. albidus* seems to be a useful method if monitoring of selenate or selenite pollution is the task.

Differences in toxicity of selenate and selenite were found on invertebrates in other cases. Selenite proved to be more toxic than selenate in *Daphnia magna* (Crustacea, Cladocera) (Johnston 1987), *Corophium* sp. (Crustacea, Amphipoda) (Hyne et al. 2002) and larvae of *Spodoptera exigua* (Lepidoptera, Noctuidae) (Trumble et al. 1998). The opposite was true for *M. scalaris* larvae (Diptera, Phoridae) (Jensen et al. 2005) and *E. albidus* (Annelida, Enchytraeidae) (this study). Therefore, we speculated that ecotoxicity mechanisms of the inorganic forms of selenium may be different below-ground than above-ground and in water. Higher toxicity of selenate compared to selenite is explainable by the higher available concentration of selenate in the soil. This finding is in correspondence with the fact that selenate is highly mobile in soil, whereas selenite is sorbed to soil components (Coppin et al. 2006).

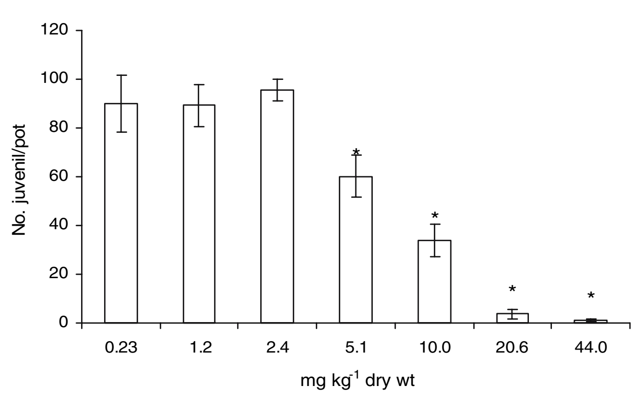

Fig. 4 Reproduction of *E. albidus* exposed to different concentrations of Na-selenite for 42 days. *Significant difference to control with $p < 0.05$. Data of the total concentrations are shown in *x*-axis

Table 6 The slope of the line fitted to the data (probit analysis) for the two test types on selenate and selenite spiked soils

	Na-selenate		Na-selenite	
	Total	available	total	available
Mortality	1.67	1.51	1.46	2.15
Reproduction	8.35	7.42	3.07	2.67

Total: $ccHNO_3 + ccH_2O_2$ soluble, available: NH_4-acetate + EDTA soluble

Acknowledgements We would like to thank Mr. József Koncz and his team for the chemical analyses and to Mrs. Zs. Bakonyi for improving the language of the text.

References

Coppin F, Chabroullet C, Martin-Garin A, Balesdent J, Gaudet JP (2006) Methodological approach to assess the effect of soil ageing on selenium behaviour: first results concerning mobility and solid fractionation of selenium. Biol Fertil Soils 42:379–386

Crommentuijn T, Doodeman CJ, Van der Pol JJ, Doornekamp A, Rademaker MC, Van Gestel CAM (1995) Sublethal sensitivity index as an ecotoxicity parameter measuring energy allocation under toxicant stress: application to cadmium in soil arthropods. Ecotoxicol Environ Saf 31:192–200

Didden WAM, Römbke J (2001) Enchytraeids as organisms for chemical stress in terrestrial ecosystems. Ecotoxicol Environ Saf 50:25–43

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

Hyne RV, Hogan AC, Pablo F, Roach AC (2002) Toxicity of selenomethionine- and seleno-contaminated sediment to the amphipod *Corophium* sp. Ecotoxicol Environ Saf 52:30–37

Jensen PD, Rivas MD, Trumble JT (2005) Developmental responses of a terrestrial insect detritivore, *Megaselia scalaris* (Loew) to four selenium species. Ecotoxicology 14:313–322

Johnston PA (1987) Acute toxicity of inorganic selenium to *Daphnia magna* (Straus) and the effect of sub-acute exposure upon growth and reproduction. Aquat Toxicol 10:335–352

Kabata-Pendias A, Adriano DC (1995) Trace metals. In: Rechcigl JE (ed) Soil amendments and environmental quality. Lewis Publishers, Boca Raton

Kádár I (1994) Importance of long-term field experiments in sustainable agriculture for Hungary. Agrokémia és Talajtan 43:291–304

Kádár I (1998) Szelén forgalma a talaj-növény rendszerben. In: Cser MÁ (eds) A szelén szerepe a környezetben és egészségvédelemben. FRAG Bt., Budapest (in Hungarian)

Lakanen E, Erviö R (1971) A comparison of eight extractants for the determination of plant available micronutrients in soil. Acta Agron Fennica 123:223–232

Lock K, Janssen CR (2001a) Tolerance changes of the potworm *Enchytraeus albidus* after long-term exposure to cadmium. Sci Total Environ 280:79–84

Lock K, Janssen CR (2001b) Ecotoxicity of mercury to *Eisenia fetida*, *Enchytraeus albidus*, and *Folsomia candida*. Biol Fertil Soils 34:219–221

Lock K, Janssen CR (2002a) Ecotoxicology of chromium (III) to *Eisenia fetida*, *Enchytraeus albidus*, and *Folsomia candida*. Ecotoxicol Environ Saf 51:203–205

Lock K, Janssen CR (2002b) Multi-generation toxicity of zinc, cadmium, copper and lead to the potworm *Enchytraeus albidus*. Environ Pollut 117:89–92

Lock K, Janssen CR (2003) Comparative toxicity of a zinc salt, zinc powder and zinc oxide to *Eisenia fetida*, *Enchytraeus albidus*, and *Folsomia candida*. Chemosphere 53:851–856

Nagy P (1999) Effects of an artificial metal pollution on nematode assemblage of a calcareous loamy chernozem soil. Plant Soil 212:35–47

Nagy P, Bakonyi G, Bongers T, Kádár I, Fábián M, Kiss I (2004) Effects of microelements on soil nematode assemblages seven years after contaminating an agricultural field. Sci Total Environ 320:131–143

OECD (2003) Guideline for testing of chemicals No. 220: Enchytraeidae reproduction test. OECD, Paris

Oldfield JE (1999) Selenium world atlas. Selenium–Tellurium Development Association, Grimbergen, Belgium

Römbke J (2003) Ecotoxicological laboratory tests with enchytraeids: a review. Pedobiologia 47:607–616

Somogyi Z, Bakonyi G, Kiss I (2005) Effects of microelements in calcareous loamy chernozem soil on *Enchytraeus albidus* under laboratory conditions. Proc Estonian Acad Sci 54:331–334

ToxRat (2003) Toxicity response analysis and testing—software (light version, 2.08). ToxRat Solutions GmbH

Trumble JT, Kund GS, White KK (1998) Influence of form and quantity of selenium on the development and survival of an insect herbivore. Environ Pollut 101:175–182

Wu L (2004) Review of 15 years of research on ecotoxicology and remediation of land contaminated by agricultural drainage sediment rich in selenium. Ecotoxicol Environ Saf 57:257–269