



(This is a sample cover image for this issue. The actual cover is not yet available at this time.)

This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at SciVerse ScienceDirect

European Journal of Soil Biology

journal homepage: <http://www.elsevier.com/locate/ejsobi>

Original article

Comparative toxicity of the selenate and selenite to the potworm *Enchytraeus albidus* (Annelida: Enchytraeidae) under laboratory conditionsZoltán Somogyi^a, Imre Kádár^b, István Kiss^a, Tünde Juríková^c, Ladislaus Szekeres^c, Štefan Balla^c, Péter Nagy^a, Gábor Bakonyi^{a,*}^aSzent István University, Department of Zoology and Animal Ecology, 2100 Gödöllő, Páter K. u. 1, Hungary^bResearch Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, H-1025 Budapest, Herman O. u. 15, Hungary^cConstantine the Philosopher University in Nitra, Institute of Natural and Informatics Sciences, Dražovská 4, 949 01 Nitra, Slovakia

ARTICLE INFO

Article history:

Received 30 September 2011

Received in revised form

16 February 2012

Accepted 21 February 2012

Available online 6 March 2012

Handling editor: Bryan Griffiths

Keywords:

Soil

Selenite

Selenate

Enchytraeus albidus

Toxicity

Bioavailability

ABSTRACT

Selenium soil chemistry is complex. It is dominated by selenates, selenites and selenides. Selenate seems to be more toxic for soil animals than selenite. However, bioavailability of different selenium forms as selenate and selenite on soil animals is poorly known. In order to investigate whether higher toxicity of selenate over selenite is a stable phenomenon to the potworm *Enchytraeus albidus*, standard laboratory tests were conducted on a chernozem brown forest soil and on a meadow chernozem. Toxicity was expressed in terms of adult mortality (LC₅₀) and juvenile production (EC₅₀). Selenate toxicity, expressed on adult mortality and juvenile production, was more substantial than that of selenite if total (conc. HNO₃ + conc. H₂O₂ soluble) concentrations were considered. No such difference was observed in the case of available (NH₄-acetate + EDTA soluble) concentrations. *E. albidus* proved to be more sensitive to selenate and selenite status of the soil than any other animal species tested before. Soil pH between 5.8 and 7.6 did not influence toxicity. The toxicity of selenate and selenite is reverse in aquatic and soil environment in most cases. The reason for this needs further investigations.

© 2012 Elsevier Masson SAS. All rights reserved.

1. Introduction

Concentrations of selenium in soils are greatly variable, typically ranging from 0.01 to 2 mg Se/kg (dry wt), but concentrations in excess of 1200 mg Se/kg (dry wt) have been reported in some areas in Ireland [1,2]. The selenium concentrations in Hungarian soils typically range from 0.1 to 2 mg/kg (dry wt). The greatest concentrations up to 5 mg/kg have been found in soils from the Bükk Mountains [3]. Most soils in Slovakia contain from 0.1 to 0.2 mg/kg (dry wt) total selenium, but concentrations between 1 and 6 mg/kg (dry wt) are also common [4]. There is a narrow range between essential and toxic concentrations of Se for living components of the ecosystems and for human health. Consequently, it is crucial to identify ecotoxicologically relevant threshold levels in soils in order to prevent excessive biomagnification of Se in food chains [5]. A selenium sensitive taxon could help with the biological monitoring of the Se status in soils.

The two common inorganic forms of selenium (selenate and selenite) seem to be differently toxic in aquatic and soil habitats.

Selenite is generally more toxic than selenate in aquatic environment [6,7]. On the other hand, selenate proved to be more toxic than selenite to soil animals [8] except in the case of *Lumbricus terrestris* [9]. The effect of selenate on *Enchytraeus albidus* mortality was 2–5 times greater than that of selenite and on juvenile production was 9–18 times greater than that of selenite [8].

There is little information available from chronic laboratory tests, which is available about the selenium toxicity to soil animals. The LC₅₀ values were as high as 258 mg/kg and 392 mg/kg (wet wt) for selenate and selenite, respectively, for the soil inhabiting larvae of the small fly *Megaselia scalaris* [10]. Acute toxicity of selenate and selenite in the earthworm *L. terrestris* was investigated by Serda and Furst [9]. The LD₅₀ values of selenate and selenite proved to be 60 and 31 mg/kg (wet wt), respectively. 50 mg/kg (wet wt) sodium selenite did not influence the mortality of the earthworm *Eisenia fetida*, but decreased juvenile production of the adults and mass gain of juveniles [11]. Toxicity values published by the above mentioned authors are higher than those found in the case of the potworm *E. albidus* [8]. A low EC₅₀ (juvenile production) value of 0.41 mg/kg (dry wt) for total Se and 0.28 mg/kg (dry wt) for available Se was found. These data were obtained on a calcareous loamy chernozem soil spiked with different concentrations of

* Corresponding author. Tel.: +36 28 522 085; fax: +36 28 410 804.

E-mail address: bakonyi.gabor@mkk.szie.hu (G. Bakonyi).

Na-selenate. In another study the LOEC of available Se for this potworm reproduction was 2 mg/kg (dry wt) after seven years of spiking with 270 mg/kg (dry wt) Se (in a form of $\text{Na}_2\text{O}_3\text{Se} \cdot 5\text{H}_2\text{O}$ application) of an experimental field in Hungary [12]. Therefore the potworm *E. albidus* seems to be sensitive to selenium status of soils.

Although the potworm *E. albidus* is a common target of ecotoxicological tests in the laboratory [13,14], it is only recently that the testing of selenium toxicity on this species has been undertaken. Somogyi et al. [8,12] found that selenium was approximately 10-times more toxic to *E. albidus* than heavy metals like cadmium [15], mercury [16], chromium (III) [17], zinc, copper and lead [18]. Reproduction proved to be a more sensitive endpoint than mortality.

Physical, chemical and biological characteristics of the soil may have significant influence on the toxicity test results. In the case of *E. albidus* pH was found of primarily importance, but other properties such as organic matter and clay content, water holding capacity, cation exchange capacity and carbon:nitrogen ratio had also an effect on pesticide test results [19,20]. pH and soil organic matter proved to be of primarily importance to copper and lead toxicity to the potworm *E. albidus* [21]. It was found in a previous experiment that selenate is considerably more toxic than selenite to *E. albidus* in a calcareous loamy chernozem soil [8]. The aim of this research was (i) verifying different toxicity of the selenate and selenite on *E. albidus* in two soil types and (ii) detecting whether total (conc. HNO_3 + conc. H_2O_2 soluble) and available (NH_4 -acetate + EDTA soluble) selenate and selenite have similar toxic effects. To clarify the transformations of inorganic selenium in soil, Na-selenate and Na-selenite transformations in soil without *E. albidus* during the 42 day experimental period were also followed in a separate experiment.

2. Materials and methods

A chernozem brown forest soil was collected from the top 20 cm of control plots at the experimental fields of the Agricultural Research Institute “Fleischmann Rudolf” of the Károly Róbert University (designated as Kompolt soil, KO) and a meadow chernozem from the top 20 cm of the control plots at the Research Institute of Karcag (Debrecen University), designated as Karcag soil (KA). Some parameters of the soils are presented in Table 1. Parameters were determined according to the Hungarian standard as follows: soil texture according to MSZ-08-0205:1978 [22], pH and CaCO_3 according to MSZ-08-0206-2:1978 [23] and organic carbon according to MSZ-08-0210:1977 [24]. Water holding capacity was determined in line with Schinner et al. [25]. Soil samples were air-dried in the laboratory, sieved on a mesh size of 2 by 2 mm and stored at 4 °C in polyethylene bags.

Concentrations of some heavy metals were tested as well. The concentrations of Ni, Cu, Pb, Zn, Cd, and Hg were below the threshold level presented by Huber et al. [26], Table 5 and in the case of As, Co and Cr according to the Hungarian regulation [27] in both soils.

Experimental soils were spiked with aqueous solutions of either Na-selenate ($\text{Na}_2\text{O}_4\text{Se}$, Sigma–Aldrich, No. 71948) or Na-selenite ($\text{Na}_2\text{O}_3\text{Se} \cdot 5\text{H}_2\text{O}$, Merck, No. 106607) at room temperature. After

24–48 h soils were stored at 4 °C for up to one week before analysis. The Se concentration of the soils was determined using a Jobin-Yvon Ultrace 238 ICP-OES spectrometer in duplicate. The conc. HNO_3 + conc. H_2O_2 soluble (referred later as “total”) concentrations were measured by using the Hungarian standard MSZ: 21470-50 [28], while NH_4 -acetate + EDTA soluble (referred later as “available”) concentrations were determined in line with Lakanen and Erviö [29]. Selenate and selenite concentrations of the control soils were below the detection limit (0.12 and 0.6 mg/kg for total and available concentrations, respectively) in both soils.

Two experiments were performed. The first experiment was a methodological one. In this case 500 g of both soil types were spiked either with selenate or selenite in three different concentrations as explained above. Nominal concentrations were as follows: 25, 50, 100 and 30, 60, 120 mg/kg for selenate and selenite, respectively. The soils were watered to 55–60% of their water holding capacity with Na-selenate or Na-selenite solutions. The soil was incubated in closed vessels at 18 ± 0.8 °C in total darkness for 42 days, the length of standard laboratory test of *E. albidus*. At two occasions, 48 h and 42 days after spiking, two soil samples of approximately 10 g each were collected from each treatment and total and available Se concentrations were determined with ICP-OES spectrometer as described above. All 10 g soil was composed from 5 subsamples taken using a soil corer of 1 cm in diameter.

In the second experiment six different selenium concentrations were prepared. Nominal concentrations for KO soil were as follows: 0.94, 1.88, 3.79, 7.5, 15, 30 and 4.7, 9.38, 18.75, 37.5, 75, 150 mg/kg for selenate and selenite, respectively. Nominal concentrations for KA soil were as follows: 3.38, 6.75, 12.5, 25, 50, 100 and 6.25, 12.5, 25, 50, 100, 150 mg/kg for selenate and selenite, respectively. Test concentrations were based on previous observations [12]. One bulk soil sample of approximately 10 g (2 g from each replicates per concentration) was collected from each treatment and total and available Se concentrations were determined with ICP-OES spectrometer as described above. Experiments with selenate and selenite spiked soils were performed separately. The animals originated from the stock cultures of the Department of Zoology and Animal Ecology, Szent István University (Gödöllő, Hungary). The culture is kept according to Römbke and Moser [14].

The test was undertaken according to the OECD guideline No. 220 [30] with minor modifications. Briefly, for the tests 150 ml glass vessels were filled with 20 g of either KO or KA oven dry soil. The soils were watered to 55–60% of their water holding capacity with Na-selenate or Na-selenite solutions. Thereafter ten adult worms with fully developed clitellum and visible eggs were put in each test vessel. The vessels were covered and kept at 18 ± 0.8 °C and $80 \pm 5\%$ RH in total darkness for 42 days. The optimum temperature for growth and reproduction of *E. albidus* is between 15 and 21 °C [31]. That is why the tests were performed at 18 ± 0.8 °C instead of 20 ± 0.8 °C as the guideline No. 220 [30] suggests. The animals were inspected once a week to check their health status (when the soil was mixed) and the soil water content. The animals were fed with small grained oak flakes (approximately 25 mg per vessel) each week. Food and the soil were gently mixed in order to reduce fungal growth and aerate it. After 42 days the vessels were sampled destructively. Mortality of the adults and reproduction (as number of juveniles living at the end of the test) were measured as end points. In order to arrange a low coefficient of the variation (CV%) of the data, ten replicates were used in the case of control and five per concentration, instead of eight (control) and four (treatments) as the guideline [30] suggests.

Data were analysed by the ToxRat [32] statistical software. LC_{50} , LC_{10} , EC_{50} and EC_{10} (juvenile production), as well as NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration) were calculated for both mortality and juvenile

Table 1

Main properties of the studied soils in ploughed layer (top 20 cm). KO: Kompolt (chernozem brown forest soil, acid clayey loam), KA: Karcag (meadow chernozem, acid clayey loam).

Soils	Sand (%)	Silt (%)	Clay (%)	WHC (%)	pH(H_2O)	CaCO_3 (%)	Organic carbon content (%)
KO	5	49	46	44	5.8	—	2.7
KA	—	53	47	47	6.8	2.0	2.9

Table 2

Total and available (\pm SD) selenate and selenite concentrations in KO (Kompolt) and KA (Karcag) soils. F: measured on the first day, L: measured on the last day. L/K: first day data per last day data *100 (%). Nominal concentrations were as follows: 25, 50, 100 and 30, 60, 120 mg/kg for selenate and selenite, respectively. Control data are not shown, because they are below detection limit (0.6 and 0.12 mg/kg for total and available concentrations, respectively).

Na-selenate						Na-selenite					
Total			Available			Total			Available		
F	L	L/F (%)	F	L	L/F (%)	F	L	L/F (%)	F	L	L/F (%)
KO soil											
23.6 (\pm 0.8)	23.0 (\pm 0.4)	97.5 (\pm 5.0)	23.6 (\pm 0.7)	18.5 (\pm 0.3)	78.4 (\pm 1.2)	34.7 (\pm 0.5)	34.2 (\pm 1.4)	98.6 (\pm 2.7)	3.46 (\pm 0.01)	3.10 (\pm 0.1)	89.6 (\pm 1.4)
49.1 (\pm 0.6)	45.3 (\pm 0.9)	92.3 (\pm 0.7)	48.9 (\pm 0.8)	39.8 (\pm 3.1)	81.4 (\pm 5.1)	68.9 (\pm 1.5)	66.6 (\pm 0.1)	96.7 (\pm 2.5)	9.88 (\pm 0.01)	7.59 (\pm 0.2)	76.8 (\pm 2.5)
100.3 (\pm 1.0)	91.2 (\pm 1.6)	90.9 (\pm 2.5)	93.6 (\pm 0.8)	85.6 (\pm 2.6)	91.5 (\pm 3.6)	142.5 (\pm 2.1)	129.0 (\pm 4.2)	90.5 (\pm 4.3)	28.4 (\pm 0.01)	18.7 (\pm 2.5)	65.9 (\pm 8.7)
KA soil											
24.4 (\pm 0.1)	21.9 (\pm 1.0)	89.8 (\pm 3.5)	24.0 (\pm 0.4)	17.0 (\pm 15.1)	70.8 (\pm 0.8)	31.3 (\pm 0.8)	26.1 (\pm 0.5)	83.4 (\pm 3.4)	13.1 (\pm 0.5)	6.1 (\pm 0.2)	46.6 (\pm 3.3)
51.2 (\pm 0.9)	46.9 (\pm 2.4)	91.6 (\pm 3.2)	48.4 (\pm 2.0)	39.8 (\pm 6.3)	82.2 (\pm 1.9)	62.9 (\pm 0.2)	51.3 (\pm 3.0)	81.6 (\pm 5.0)	32.2 (\pm 0.3)	13.0 (\pm 0.3)	40.4 (\pm 0.5)
102.7 (\pm 2.2)	93.6 (\pm 3.3)	91.1 (\pm 1.2)	98.5 (\pm 1.6)	84.1 (\pm 4.8)	85.4 (\pm 0.5)	136.1 (\pm 1.8)	118.7 (\pm 1.3)	87.2 (\pm 0.3)	80.2 (\pm 1.1)	20.6 (\pm 1.1)	25.7 (\pm 1.0)

production. The homogeneity of the data was tested by Cochran's test. In the case of homogeneous variances, *t*-test and Williams multiple sequential *t*-test were calculated. Data with non-homogeneous variances were analysed by Welch *t*-test or by Welch *t* with Bonferroni adjustment. Probit analysis was based on linear maximum likelihood regression. The method of Litchfield and Wilcoxon [33] was applied to calculate slope value as follows: $S = ((LC_{84}/LC_{50}) + (LC_{50}/LC_{16}))/2$, where *S* = the slope value. The slope values were used as estimators of the sensitivity of *E. albidus* reactions (mortality or juvenile production) to the changes of the selenium concentrations. Differences of juvenile production in control vessels were compared by repeated-ANOVA after transforming data by $\ln + 1$.

3. Results

In the first experiment different behaviour of selenate and selenite was observed in the KO and KA soils. In the KO soil 91–99% of the spiked selenate and selenite were found as total at the end of the experiment (Table 2) showing that only a small fraction of the selenium volatilised during the experimental period. Somewhat less selenate and selenite was recovered in the available form (66–92%). Greater differences between the concentrations measured at the start and at the end of the experiment were found in the KA soil. 90–92% and 82–87% of selenate and selenite, respectively, were detected as total selenium at the end of the experiment (Table 2). The available form was lower—71–85% and 26–47% in the case of selenate and selenite, respectively—compared to the concentration at spiking. This suggests higher adsorption ability of the KA soil.

The behaviour of Na-selenate and Na-selenite in soil turned out to be as expected in the second experiment. Na-selenate was

weakly bound to the soil particles and more than 90% of it remained in available form (95–98% and 92–99% in KO and KA soils, respectively) at the start of the experiment (Table 3). On the other hand, available form of the Na-selenite was relatively little compared to the total concentration (7–22% and 30–59% in KO and KA soils, respectively) (Table 3). Adult mortality and reproduction met or exceeded the requirements of the OECD test guidelines in all experiments, because mortality was less than 20% and juvenile production was more than 25 per test vessel in every control soil (the lowest number was 27.4 and the highest 114.8 per vessel) [30]. Juvenile production in the control vessels of the KO soil was 27.4 (\pm 5.7) and 32.2 (\pm 8.0) individuals (\pm SE) per vessel in the selenate and selenite experiments (carried out at different dates), respectively. In the case of the KA soil 93.1 \pm 49.8 and 114.8 \pm 46.9 juvenile individuals (\pm SE) per vessel were found in the control vessels of the selenate and selenite experiments, respectively. The juvenile production in the control vessels was significantly influenced by the soil types (*F*: 33.3, *p* < 0.001) and the date when the experiment was carried out (*F*: 4.5, *p* < 0.05).

Selenate toxicity of *E. albidus* in KO soil was detected at the lowest concentration tested (second experiment). Consequently no NOEC determination is possible in this case. The LOEC for both adult mortality and juvenile production was 2.9 mg/kg dry wt (Fig. 1). Selenite was considerably less toxic to *E. albidus* in this soil. The NOEC for adult mortality and juvenile production was 8.9 mg/kg dry wt and 3.9 mg/kg dry wt, respectively (Fig. 2). The corresponding values of LOEC for mortality and juvenile production were 16.8 mg/kg dry wt and 8.9 mg/kg dry wt, respectively.

In the KA soil spiked with selenate the NOEC value for adult mortality was observed at 3.4 mg/kg dry wt. A significant increase in mortality was recorded in soils containing 5.8 mg/kg dry wt

Table 3

Total and available concentrations of Se in Na-selenate and Na-selenite spiked KA and KO soils, and ratio of available and total selenate and selenite concentrations as percent (%). KO: Kompolt, KA: Karcag, T: Total, A: available, A/T: available per total *100 (%). Total: conc. HNO₃ + conc. H₂O₂ soluble, available: NH₄-acetate + EDTA soluble. Nominal concentrations for KO soil were as follows: 0.94, 1.88, 3.79, 7.5, 15, 30 and 4.7, 9.38, 18.75, 37.5, 75, 150 mg/kg for selenate and selenite, respectively. Nominal concentrations for KA soil were as follows: 3.38, 6.75, 12.5, 25, 50, 100 and 6.25, 12.5, 25, 50, 100, 150 mg/kg for selenate and selenite, respectively. Control data are not shown, because they are below detection limit (0.6 and 0.12 mg/kg for total and available concentrations, respectively).

KO soil						KA soil					
Na-selenate			Na-selenite			Na-selenate			Na-selenite		
T	A	A/T (%)	T	A	A/T (%)	T	A	A/T (%)	T	A	A/T (%)
2.92	2.79	95.6	3.88	0.27	7.0	1.66	1.55	93.4	8.39	2.50	29.8
6.14	6.04	98.4	8.89	0.61	6.9	3.42	2.56	74.9 ^a	17.1	5.94	34.7
12.5	11.8	94.7	16.8	1.42	8.5	5.77	5.33	92.4	31.3	13.1	41.8
25.6	25.1	98.1	33.2	3.37	10.2	12.9	12.4	95.7	62.9	32.2	51.1
49.5	48.4	97.7	70.7	10.2	14.4	24.8	24.5	98.8	107.7	59.3	55.0
101.5	98.5	97.1	144.5	31.7	21.9	31.9	31.5	98.8	136.1	80.2	58.9

^a Outlier data based on Grubbs test (*z*: 1.89, *p* < 0.05) [48].

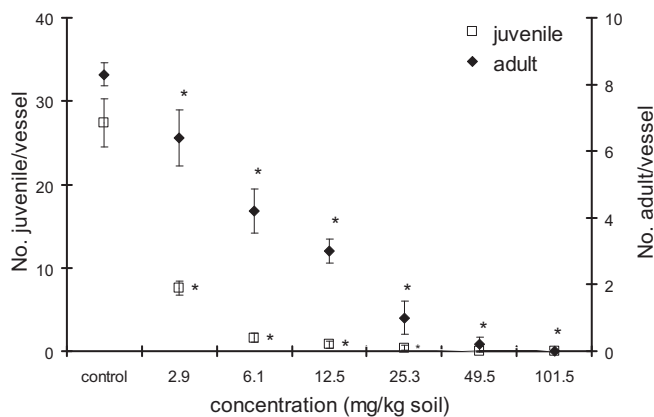


Fig. 1. Mortality and reproduction of *E. albidus* exposed to different concentrations of Na-selenate on Kompolt (KO) soil for 42 days. Values are expressed as average (\pm SE). *Significant difference to control at least $p < 0.05$. Data of the total concentrations are shown in x-axis. Control: below detection limit. Concentrations are expressed on dry weight basis.

(LOEC) and above (Fig. 3). In the case of juvenile production, the highest numbers were found at 3.4 mg/kg dry wt concentration, although this increase was not significantly different in comparison with the control sample. No such response in juvenile numbers to low selenium concentration was observed in the KO soil. The NOEC value for both adult mortality and juvenile production in selenite spiked soil was 8.4 mg/kg dry wt, while a significant increase in adult mortality and a decrease in juvenile production were observed at 17.4 mg/kg dry wt (LOEC) (Fig. 4).

No noticeable differences were found in selenate spiked soils either between LC_{50} or EC_{50} (juvenile production) values if the data were calculated for total and available selenate concentrations (Table 4). LC_{50} data of selenate spiked soils are very similar. Adult mortality values are very close to each other in both soils, but juvenile production was influenced by soil types. Besides, juvenile production was about 4 and 1.4 times more sensitive an endpoint in KO and KA soils, respectively, than the corresponding adult mortality. The EC_{50} (juvenile production) values indicate that the toxic threshold for selenate in the KO soil was lower than that for the KA soil.

LC_{50} and EC_{50} (juvenile production) values for total selenite concentrations were considerably higher than those for selenate for both soil types (Table 4). No such differences were observed for available

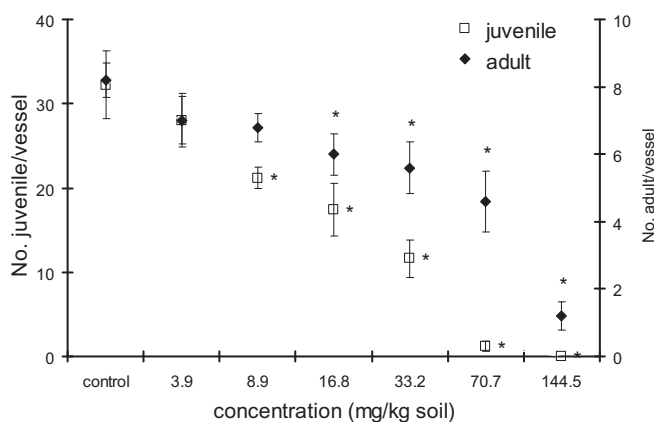


Fig. 2. Mortality and reproduction of *E. albidus* exposed to different concentrations of Na-selenite on Kompolt (KO) soil for 42 days. Values are expressed as average (\pm SE). *Significant difference to control at least $p < 0.05$. Data of the total concentrations are shown in x-axis. Control: below detection limit. Concentrations are expressed on dry weight basis.

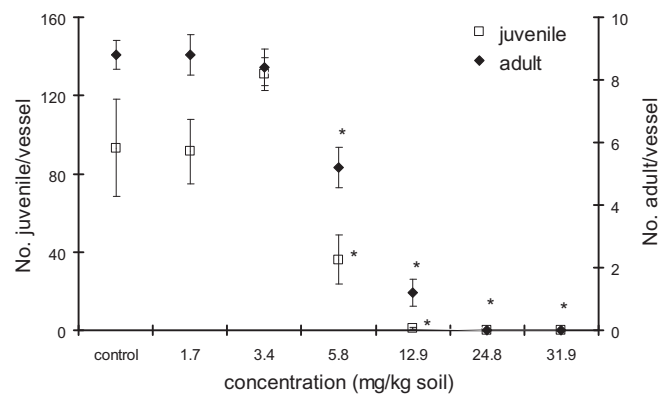


Fig. 3. Mortality and reproduction of *E. albidus* exposed to different concentrations of Na-selenate on Karcag (KA) soil for 42 days. *Significant difference to control at least $p < 0.05$. Data of the total concentrations are shown in x-axis. Control: below detection limit. Concentrations are expressed on dry weight basis.

LC_{50} and EC_{50} (juvenile production) data. Juvenile production was by 3.2 and 1.4 (total) and 4.9 and 1.5 (available) times more sensitive an endpoint in KO and KA soil, respectively, than adult mortality. Selenate toxicity for reproduction was lower in KA than in KO soil when total as well as available concentrations were considered. The same figure was observed in the case of available concentrations, but no such relationship was detected between these soil parameters and toxicity in the case of selenite if total concentrations were considered (Table 4).

The slope values of the dose–response regression curves were higher in the KO soil than those in KA soil in every case (Table 5). This means that one unit alteration in selenate or selenite concentration generates more change in adult mortality or juvenile production in KO soil than in KA soil.

4. Discussion

Adsorption of selenate and selenite in soil is influenced by several factors [34]. Comparative analyses conducted in similar types of soils show that selenite adsorption to the solid soil phase is greater than that of selenate under similar conditions [35,36]. This was the case in this study as well. Therefore it was supposed that substantial transformation of selenium forms did not occur during the experiments. Selenium sorption generally decreases with an increasing pH [37]. In contrast to this trend, in our study stronger

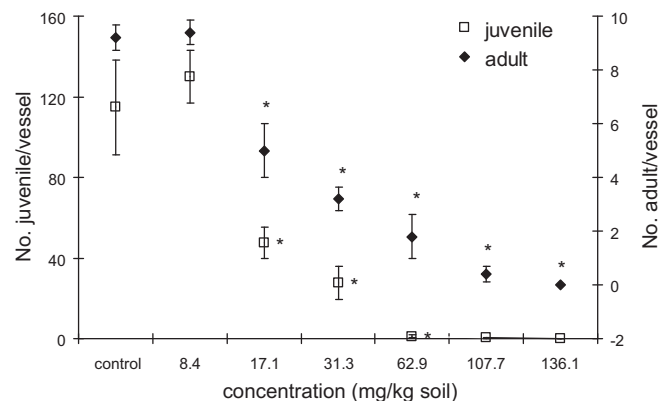


Fig. 4. Mortality and reproduction of *E. albidus* exposed to different concentrations of Na-selenite on Karcag (KA) soil for 42 days. *Significant difference to control at least $p < 0.05$. Data of the total concentrations are shown in x-axis. Control: below detection limit. Concentrations are expressed on dry weight basis.

Table 4

Summary of toxicity benchmarks for Se determined in tests with *E. albidus* using Na-selenate and Na-selenite spiked soils. Adult mortality means LC₅₀, while juvenile production EC₅₀. R²: coefficient of determination. Values are expressed as mg/kg dry wt. 95% confidence limits are in parentheses. Total: conc. HNO₃ + conc. H₂O₂ soluble, available: NH₄-acetate + EDTA soluble. KO: Kompolt, KA: Karcag. n.d.: non determined.

	Adult mortality				Juvenile production			
	Total		Available		Total		Available	
	LC ₅₀	R ²		R ²	EC ₅₀	R ²		R ²
Selenate								
KO	6.9 (5.4–8.7)	0.97	6.7 (5.4–8.2)	0.98	1.8 (0.5–2.4)	0.95	1.7 (0.6–2.2)	0.96
KA	6.8 (5.3–9.9)	0.96	6.2 (5.5–7.1)	0.99	5.0 (n.d.)	0.99	4.6 (n.d.)	0.99
Selenite								
KO	55.1 (26.1–219.4)	0.79	7.4 (3.1–30.9)	0.84	17.1 (10.5–27.6)	0.92	1.5 (0.9–2.6)	0.93
KA	19.8 (11.8–26.0)	0.98	7.2 (3.2–10.7)	0.97	14.6 (0.1–20.9)	0.92	4.9 (0–7.8)	0.91

sorption of available selenite was detected in KA soil which was characterised by somewhat higher pH. This may be because higher microbial activity enhances selenite incorporation in humic substance fractions [38,39].

According to the LC₅₀ and EC₅₀ (juvenile production) values for both total selenate and selenite spiking proved to be more toxic by at least an order of magnitude to *E. albidus* than heavy metals [15,17,18], and the herbicide Phenmedipham [19,20]. Selenium toxicity to *E. albidus* is greater than that to other soil animals like the earthworm *E. fetida* [11], *L. terrestris* Serda and Furst [9] and the larvae of the fly *M. scalaris* [10,40]. Moreover the differences in slope values of the dose–response regression curves stress the fact that the distance of necessary and toxic concentrations of selenium depends on the soil type having a narrower range in more acid soil. This is important considering the narrow range between essential and toxic concentrations of Se.

Our findings indicate marked differences in measured toxic threshold between selenate and selenite for *E. albidus*, when total concentrations are considered, with the former being more toxic than the latter under the experimental conditions employed. These findings corroborate those of other studies on soil invertebrates [8,10], except for *L. terrestris* [9], but in contrast with those reported for the aquatic amphipods *Hyalella azteca* [41] and *Corophium* sp. [42], the waterflea *Daphnia magna* [43–45], and two terrestrial moth larvae *Spodoptera exigua* [46] and *Heliothis virescens* [47]. It has also been suggested by Eisler [7] as a general phenomenon that selenite is more toxic than selenate in the aquatic environment.

The fact that the difference in toxicity between either selenium forms disappears when expressed as “available” concentration suggests different uptake routes of selenate and selenite through body surface and gut. Selenium (both selenate and selenite) uptake through the body surface is possible merely from pore water. The effect which is generated by available concentration in pore water is expected in this case. On the other hand the food of *E. albidus* contains pore water, organic matter and inorganic soil particles,

which include total soil selenium concentration. We suggest the hypothesis that the gut environment (enzymes, gut microbes) makes the selenium uptake possible both from liquid (pore water) and solid soil compartments. Furthermore it is presumed that selenite is less available from gut than selenate because it is effectively bound to soil solid phase [36], but selenate is much more mobile. Both hypotheses are worthy for future investigations.

In general, enchytraids show high sensitivity to differences in soil pH [20]. Amorim et al. [19] found significant correlation between pH and adult mortality and juvenile production if the range of pH was from 3.2 to 7.4. No such relationship was found in the case of the adult mortality in this study. Both available selenate and selenite were more toxic for juvenile production in KO soil having lower pH compared to KA soil. However, both selenite and selenate had higher toxicity on juvenile production in the soil of our previous experiment [8] having pH_(H2O) as high as 7.6 (not published data). Therefore pH dependence does not seem to be a reasonable explanation for different toxicity of selenite and selenate between soil pH 5.8 and 7.6. This may occur because most deleterious effects on mortality are to be found if pH is below 5 and other soil factors such as organic matter have an influence on toxicity [19].

5. Conclusions

E. albidus proved to be more sensitive to inorganic selenium (selenate and selenite) status of the soil than any other animal species tested before. Juvenile production is a suitable endpoint to assess soil selenium concentration and small changes of it. Therefore this endpoint is emphasized for standard laboratory tests. Bioavailability of selenate and selenite seems to be different. This may be the explanation why distinct toxic effects of selenate and selenite were observed in the case of the total, but not for the available concentrations.

Acknowledgements

We would like to thank Mr. József Koncz and his team for the chemical analyses and Mrs. Zs. Bakonyi for improving the language of the text. Research was supported by the TÁMOP-4.2.2.B-10/1 “Development of a complex educational assistance/support system for talented students and prospective researchers at the Szent István University” project.

References

- [1] H.F. Mayland, L.F. James, K.E. Panter, J.L. Sonderegger, Selenium in seleniferous environment, in: L.W. Jacobs (Ed.), Selenium in Agriculture and the Environment (1989) SSSA Special Publication Number 23., Madison, Wisconsin, USA.
- [2] J.E. Oldfield, Selenium World Atlas. Selenium-Tellurium Development Association, Grimbergen, Belgium, 1999.

Table 5

The slope value of the line fitted to the data (probit analysis) for the two test types on Na-selenate and Na-selenite spiked soils. Adult mortality means slope value of line fitted to mortality data, while juvenile production means slope value of line fitted to data of juvenile numbers. Total: conc. HNO₃ + conc. H₂O₂ soluble, available: NH₄-acetate + EDTA soluble. KO: Kompolt, KA: Karcag.

Soils	Adult mortality		Juvenile production	
	Total	Available	Total	Available
Selenate				
KO	3.31	3.31	2.27	2.35
KA	1.69	1.82	1.58	1.6
Selenite				
KO	5.57	10.67	3.49	4.95
KA	3.30	4.60	2.47	3.23

- [3] I. Kádár, Selenium cycle in soil-plant systems, in: M.Á. Cser (Ed.), Selenium in the Environment and Health, FRAG Bt., Budapest, 1998 (in Hungarian).
- [4] O. Hegedűs, A. Hegedűsová, S. Šimková, Selenium as Biogen Element, UKF, Nitra, 2007.
- [5] L. Wu, Review of 15 years of research on ecotoxicology and remediation of land contaminated by agricultural drainage sediment rich in selenium, *Ecotox. Environ. Safety* 57 (2004) 257–269.
- [6] S.P. Canton, Acute aquatic life criteria for selenium, *Environ. Toxicol.* 18 (1999) 1425–1432.
- [7] R. Eisler, *Eisler's Encyclopedia of Environmentally Hazardous Priority Chemicals*, Elsevier, Amsterdam, 2007.
- [8] Z. Somogyi, I. Kiss, I. Kádár, G. Bakonyi, Toxicity of selenate and selenite to the potworm *Enchytraeus albidus* (Annelida: Enchytraeidae): a laboratory test, *Ecotoxicology* 16 (2007) 379–384.
- [9] S. Serda, A. Furst, Acute toxicity of selenium to earthworms, *Proc. Western Pharm. Soc.* 30 (1987) 277–278.
- [10] P.D. Jensen, M.D. Rivas, J.T. Trumble, Developmental responses of a terrestrial insect detritivore, *Megaselia scalaris* (Loew) to four selenium species, *Ecotoxicology* 14 (2005) 313–322.
- [11] E. Fischer, L. Koszorus, Sublethal effects, accumulation capacities and elimination rates of As, Hg and Se in the manure worm, *Eisenia fetida* (Oligochaeta, Lumbricidae), *Pedobiologia* 36 (1992) 172–178.
- [12] Z. Somogyi, G. Bakonyi, I. Kiss, Effects of microelements in calcareous loamy chernozem soil on *Enchytraeus albidus* under laboratory conditions, *Proc. Estonian Acad. Sci.* 54 (2005) 331–334.
- [13] J. Römbke, Ecotoxicological laboratory tests with enchytraeids: a review, *Pedobiologia* 47 (2003) 607–616.
- [14] J. Römbke, Th. Moser, Validating the enchytraeid reproduction test: organisation and results of an international ringtest, *Chemosphere* 46 (2002) 1117–1140.
- [15] K. Lock, C.R. Janssen, Tolerance changes of the potworm *Enchytraeus albidus* after long-term exposure to cadmium, *Sci. Total Environ.* 280 (2001a) 79–84.
- [16] K. Lock, C.R. Janssen, Ecotoxicity of mercury to *Eisenia fetida*, *Enchytraeus albidus*, and *Folsomia candida*, *Biol. Fertil. Soils* 34 (2001b) 219–221.
- [17] K. Lock, C.R. Janssen, Ecotoxicology of chromium (III) to *Eisenia fetida*, *Enchytraeus albidus*, and *Folsomia candida*, *Ecotox. Environ. Safety* 51 (2002) 203–205.
- [18] K. Lock, C.R. Janssen, Multi-generation toxicity of zinc, cadmium, copper and lead to the potworm *Enchytraeus albidus*, *Environ. Pollut.* 117 (2002) 89–92.
- [19] M.J.B. Amorim, J. Römbke, A. Scheffczyk, A.M.V.M. Soares, Effect of different soil types on the enchytraeids *Enchytraeus albidus* and *Echytraeus luxuriosus* using the herbicide Phenmedipham, *Chemosphere* 61 (2005) 1102–1114.
- [20] R.G. Kuperman, M.J.B. Amorim, J. Römbke, R. Lanno, R.T. Checkai, S.G. Dodard, G.I. Sunahara, A. Scheffczyk, Adaptation of the enchytraeid toxicity test for use with natural soil types, *Eur. J. Soil Biol.* 42 (2006) 234–243.
- [21] K. Lock, C.R. Janssen, Test designs to assess the influence of soil characteristics on the toxicity of copper and lead to the Oligochaete *Enchytraeus albidus*, *Ecotoxicology* 10 (2001) 137–144.
- [22] MSZ-08–0205:1978, Determination of Physical and Hydrophysical Properties of Soils, Hungarian Standard Institution, Budapest, 1978, (in Hungarian).
- [23] MSZ-08–0206–2:1978, Evaluation of Some Chemical Properties of the Soil. Laboratory Tests. (pH Value), Phenolphthaleine Alkalinity Expressed in Soda, All Water Soluble Salts, Hydrolite (y1-value) and Exchanging Acidity (y2-Value), Hungarian Standard Institution, Budapest, 1978, (in Hungarian).
- [24] MSZ-08–0210:1977, Testing Organic Carbon Content in Soils, Hungarian Standard Institution, Budapest, 1977, (in Hungarian).
- [25] F. Schinner, R. Öhlinger, E. Kandeler, R. Margesin (Eds.), *Bodenbiologische Arbeitsmethoden*, Springer-Verlag, Berlin, Heidelberg, NewYork, 1993.
- [26] S. Huber, G. Prokop, D. Arrouays, G. Banko, A. Bispo, R.J.A. Jones, M.G. Kibblewhite, W. Lexer, A. Möller, R.J. Rickson, T. Shishkov, M. Stephens, G. Toth, J.J.H. Van den Akker, G. Varallyay, F.G.A. Verheijen, A.R. Jones (Eds.), *Environmental Assessment of Soil for Monitoring, Indicators & Criteria*. EUR 23490 EN/1, vol. I, Office for the Official Publications of the European Communities, Luxembourg, 2008.
- [27] 6/2009. (IV. 14.) KvVM-EüM-FVM Joint Decree The Soil and Groundwater Pollution Threshold Levels and the Measurement of Pollutions (2009) (in Hungarian).
- [28] MSZ 21470–50:1998, Environmental Soil Analyses. Determination of the Total and Available Toxic Elements, Heavy Metals and Chromium(VI) Concentrations, Hungarian Standard Institution, Budapest, 1998, (in Hungarian).
- [29] E. Lakanen, R. Erviö, A comparison of eight extractants for the determination of plant available micronutrients in soil, *Acta Agron. Fenn.* 123 (1971) 223–232.
- [30] OECD, Guideline for Testing of Chemicals No. 220, Enchytraeidae Reproduction Test, Paris, 2004.
- [31] I.V. Ivleva, Growth and reproduction of the potworm (*Enchytraeus albidus*), *Zool. Zh.* 32 (1953) 394–404 (in Russian).
- [32] ToxRat, Toxicity Response Analysis and Testing – Software (Light Version, 2.08), ToxRat Solutions GmbH, 2003.
- [33] J.T. Litchfield, F.A. Wilcoxon, A simplified method of evaluating dose-effect experiments, *J. Pharmacol. Exp. Ther.* 96 (1949) 99–113.
- [34] S. Hyun, P.E. Burns, I. Murarka, L.S. Lee, Selenium (IV) and (VI) sorption by soils surrounding fly ash management facilities, *Vadose Zone J.* 5 (2006) 1110–1118.
- [35] F. Coppin, C. Chabroulet, A. Martin-Garin, J. Balesdent, J.P. Gaudet, Methodological approach to assess the effect of soil ageing on selenium behaviour: first results concerning mobility and solid fractionation of selenium, *Biol. Fert. Soils* 42 (2006) 379–386.
- [36] S. Sharmasarkar, G.F. Vance, Selenite-selenate sorption in surface coal mine environment, *Adv. Environ. Res.* 7 (2002) 87–95.
- [37] K.H. Goh, T.T. Lim, Geochemistry of inorganic arsenic and selenium in a tropical soil: effect of reaction time, pH, and competitive anions on arsenic and selenium adsorption, *Chemosphere* 55 (2004) 849–859.
- [38] S. Eich-Greatorex, T.A. Sogn, A.F. Ogaard, I. Aasen, Plant availability of inorganic and organic selenium fertiliser as influenced by soil organic matter content and pH, *Nutr. Cycl. Agroecosyst.* 79 (2007) 221–231.
- [39] J.P. Gustafsson, L. Johnsson, The association between selenium and humic substances in forested ecosystems – laboratory evidence, *Appl. Organomet. Chem.* 8 (1994) 141–147.
- [40] P.D. Jensen, L.R. Johnson, J.T. Trumble, Individual and joint actions of selenate and methylmercury on the development and survival of insect detritivore *Megaselia scalaris* (Diptera: Phoridae), *Arch. Environ. Contam. Toxicol.* 50 (2006) 523–530.
- [41] A.M. Brasher, R.S. Ogle, Comparative toxicity of selenite and selenate to the amphipod *Hyalella azteca*, *Arch. Environ. Contamin. Toxicol.* 24 (1993) 182–186.
- [42] R.V. Hyne, A.C. Hogan, F. Pablo, A.C. Roach, Toxicity of Selenomethionine- and seleno-contaminated sediment to the Amphipod *Corophium* sp. *Ecotox. Environ. Safety* 52 (2002) 30–37.
- [43] A.M. Dunbar, J.M. Lazorchak, W.T. Waller, Acute and chronic toxicity of sodium selenate to *Daphnia magna* Straus, *Environ. Toxicol. Chem.* 2 (1983) 239–244.
- [44] P.A. Johnston, Acute toxicity of inorganic selenium to *Daphnia magna* (Straus) and the effect of sub-acute exposure upon growth and reproduction, *Aquat. Toxicol.* 10 (1987) 335–352.
- [45] K.J. Maier, C.G. Foe, A.W. Knight, Comparative toxicity of selenate, selenite, seleno-DL-methionine and seleno-DL-cystine to *Daphnia magna*, *Environ. Toxicol. Chem.* 12 (1993) 755–763.
- [46] J.T. Trumble, G.S. Kund, K.K. White, Influence of form and quantity of selenium on the development and survival of an insect herbivore, *Environ. Pollut.* 101 (1998) 175–182.
- [47] H.J.R. Popham, K.S. Shelby, Effect of inorganic and organic forms of selenium supplementation on development of larval *Heliothis virescens*, *Ent. Exp. App.* 125 (2007) 171–178.
- [48] G.W. Snedecor, W.G. Cochran, *Statistical Methods*, eighth ed. IowaState University Press, 1989.