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The Effect of Bauxite Residue on the Avoidance Behavior of *Enchytraeus albidus* (Enchytraeidae)

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Abstract

The potworm *Enchytraeus albidus* has been used as a testorganism to assess the effects of soil polluting chemicals. The current standardized test methods do not exploit the sensitivity of avoidance behavior of the animals towards chemicals as measurement endpoint. In this paper we tested the effect of bauxite residue (BR), the waste from alumina production, on the habitat of a macrodecomposer in three different soils: an acidic sandy soil (NY), a carbonated sandy soil (OB) and a silty soil (NH). In the acute toxicity tests the testing of whole soil samples proved to be more sensitive compared to the testing of soil suspensions and soil extracts, which indicated significant mortality only at over 50 % BR in soil. EC_{20} values were 9 % BR in OB, 28 % BR in NH and 34 % BR in NY in the whole soil tests of our study. In the avoidance test potworms chose the 1 % and 5 % BR treated soils instead of the control soils, but avoided the 10 % and 25 % BR concentrations. Besides the common methodology applied in avoidance tests, we offered not only control and treated soil pairs to the animals, but we also paired all the concentrations with each other. *E. albidus* chose 10 % BR amount in soil instead of 25 % BR in all cases. With the avoidance test we could show not only negative, but also positive effects of soil amendments: BR addition to soils at up to 5 % can result in a better habitat for potworms.

Keywords

potworm, *Enchytraeus albidus*, bauxite residue, acute toxicity, avoidance

1 Introduction

The potworms or enchytraeids are a group of citellate annelids (or microannelids) [1, 2]. Potworms have essential role in the decomposition of organic matter and nutrient cycling [3, 4]. They have an influence on air permeability, pore structure and aggregate stability in soils [5, 6]. Because of their importance in soil ecosystems, enchytraeids have been used for ecotoxicological testing in laboratory to test the effect of pesticides, metals and other chemicals [7]. Their first usage as ecotoxicological test animals was presented in 1968 [8], and nowadays there are several test methods using potworms to measure the effect of different environmental stress-factors on these animals [2]. The avoidance behavior of animals, e.g. Collembola and earthworms, can be used as measurement endpoint in ecotoxicity tests [9, 10], because it influences the energy budget of animals by dispending energy on detecting and escaping from hazard [11]. Some studies examined the effect of xenobiotics, e.g. pesticides and metals on the avoidance behavior of potworms [11, 12]. Standardized ecotoxicity tests focus on the measurement of the bioaccumulation of chemicals in potworms [13] and the effect of chemicals on their reproduction and mortality [14].

Bauxite residue (red mud) is the waste of alumina production. Typically, 0.7–2 tonnes of residue per each tonne of alumina is generated [15]. Even though each bauxite residue is different in composition, most of them contain Fe_2O_3, Al_2O_3, SiO_2, TiO_2, CaO, Na_2O and LOI (loss on ignition) as main components [16]. This material is highly saline (> 50 g L^{-1}) and alkaline (pH 10–13), which represents the main disposal problem in the alumina industry [17]. The high risk of bauxite residue deposition was demonstrated at Ajka (Hungary) [18-20]. As a reuse option the bauxite residue can be applied as a soil amendment [21, 22], or as immobilizer of metals, such as Cd, Pb and Zn, in soil [23-25]. However, in the case of such applications the environmental risks should always be assessed, which require new problem-specific ecotoxicological test methods [26].

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There are only few studies available on the ecotoxicological effects of bauxite residues on soil living animals. The environmental effects of the bauxite residue contaminated soil after the Ajka spill were assessed on-site through the Collembola fauna [27] and by laboratory acute toxicity tests with Collembola (*Folsomia candida*) [28, 29]. Maddocks [30] tested the effect of metal-loaded Bauxol reagent produced from bauxite residue on earthworm (*Eisenia fetida*), while Courtney et al. [31] examined the succession rate of macro-athropodes on recultivated bauxite residue. In this study we investigated for the first time the avoidance behavior of potworms on bauxite residue amended soils. In the standardized method with earthworms and in previous studies with potworms the examined sample was only paired with the control to test avoidance behavior (selection of a preferable area of soil over the area they escape from [2, 12]) [11, 32], but in this study we paired with each other also the soil samples amended with various bauxite residue doses to gain a more detailed picture on potworm behavior in contact with bauxite residue amended soils.

2 Materials and Methods

2.1 Materials

The bauxite residue (BR; pH = 10.4) originated from a storing area in Hungary (Table 1). The acidic sandy soil is from Nyírlugos (NY), the carbonated sandy soil from Őrbottyán (OB), and the silty soil from Nagyhörcsök (NH), Hungary (Table 2).

Table 1 Total (aqua regia extractable) and mobile (distilled water extractable) element content of BR

Element (mg/kg)	Total content[a] (mg/kg)	Mobile content[b] (mg/kg)
Al	5011	567
As	28.5	0.392
Co	39.1	< DL
Cr	256	0.148
Cu	70.4	< DL
Mo	ND	1.97
Na	27620	1792
Ni	165	< DL
Zn	43.2	< DL
V	171	4.36

ND: no data, DL: detection limit, DL_{Co} = 0.008 mg/kg, DL_{Cu} = 0.050 mg/kg, DL_{Ni} = 0.040 mg/kg, DL_{Zn} = 0.050 mg/kg
[a] Total element content measured after aqua regia digestion [33]
[b] Mobile element content measured after distilled water extraction [34]

Table 2 Soil properties

Soil properties	NH	NY	OB
Sand (%) (> 0.05 mm)[a]	17	85	81
Silt (%) (0.05–0.002 mm)[a]	60	10	13
Clay (%) (< 0.002 mm)[a]	23	5	6
Water holding capacity (%)[b]	36	30	32
Humus (%)[c]	3.1	0.5	1.0
$CaCO_3$ (%)[d]	1.8	0.0	3.3
pH(H_2O)[e]	7.6	4.9	7.7

[a] Texture [35], [b] WHC [36], [c] Humus [37], [d] $CaCO_3$ [38], [e] pH [37]

2.2 Experimental set up

We mixed BR with the soils at 1 $^w/_w$%, 5 $^w/_w$%, 10 $^w/_w$%, 25 $^w/_w$% and 50 $^w/_w$% (BR 1–50 %) and an untreated soil was used as control (C). Ujaczki et al. [22, 29] suggested to apply BR in sandy soils at up to 5 %, therefore smaller and higher amounts were tested. The soil was watered to 60 % water holding capacity and kept at 22 ± 2 °C for a week before testing.

2.3 Chemical analysis

The pH was measured in 1:2,5 soil: distilled water suspension after 30 min shaking at 160 rpm in three replicates [35]. Total element content was measured by NITON XL3t 600 in three replicates.

2.4 Sample preparation

The soils were air dried, ground and sieved through a 1 mm sieve before testing. Beyond testing direct effect of whole soils (WS), we made soil suspensions (S) and soil extracts (E) [37]. Shortly, 4.5 g of soil was put into an Erlenmeyer flask with 45 ml of distilled water (1:10 solid: liquid ratio) shaken for 4 hours at 140 rpm to make the soil suspension. Then the suspension was settled for five minutes and filtered through 4–12 μm hole-diameter filter paper to make the soil extract. The samples were stored at 5 °C until testing.

2.5 Test organisms

The samples were tested by potworm acute mortality and avoidance tests. The population of *Enchytraeus albidus* (Henle 1837) was from our own stock culture. We maintained the stock culture in potting soil at constant temperature (24 ± 1 °C) with twice a week wetting, protected from direct light. The animals were fed with ground cereal once a week. Adult, minimum 12 mm long animals were applied for testing.

2.6 Acute mortality tests

The acute mortality tests were carried out according to OECD 207 [39] modified for enchytraeids in five replicates. For the whole soil test, we measured 25 g of soil in a 9 cm glass Petri-dish wetted with 5 ml of boiled tap water, for the soil suspension and soil extract we added 3 ml of the liquid on a filter paper in similar Petri-dishes. We added 5 adult animals to each sample and kept them in the dark at 21 ± 1 °C for 96 h. The number of living animals was counted.

2.7 Avoidance tests

The avoidance tests were carried out based on Amorim et al.'s work [40], but we modified and complemented their method. Avoidance is an active selection activity of the animals between two samples with different properties [2, 12]. Instead of making pairs only between the control (C) and the treated soils (BR1–25 %), we offered the animals all the concentrations in pairs. We paired: C-C; C-BR1 %; C-BR5 %; C-BR10 %; C-BR25 %; BR1-BR5 %; BR1-BR10 %; BR1-BR25 %; BR5-BR10 %; BR5-BR25 %; BR10-BR25 %. We also paired the same BR concentrations in the various soil types with each other (NH-NY, NH-OB, NY-OB).

We introduced 20-20 g of soil onto the two sides of Petri-dishes, wetted them with 4-4 ml of boiled tap water. The tests were carried out in six replicates with four-four adult animals placed on each side of the Petri-dish. After 96 hours we counted the animals on the two sides of each Petri-dish.

2.8 Statistical analysis

We applied Pearson correlation analysis to examine correlations between BR amount (as numerical variables), and pH, element content, number of dead animals in the acute toxicity tests and number of animals at the two sides of Petri-dishes in avoidance test. We used One-way ANOVA (Fisher LSD) to determine the statistically significant differences between the means of the number of dead animals, pH and element content in each treatment. We applied one-way ANOVA (Type 1 LR test) in Poisson-distribution to compare the average number of animals at the two sides of Petri-dishes in avoidance test in case of different pairs. We applied one-way and multi-way ANOVA for the normalized data in StatSoft® Statistica 7.0 programs to investigate which factorial variables had significant effect on the number of animals at the two sides of Petri-dishes in avoidance tests. We applied 95 % confidence intervals for significant differences. The Figures were produced by Microsoft Office Excel 2013 and Allplan 2014 softwares. In the tables and diagrams the letters represent the significantly different groups in the one-way ANOVA tests. The "r" value in the tables represents the correlation coefficient. Correlation was considered very strong when r-value > **|0.85|** and strong when r-value > *|0.60|* [41]. The concentration-response curves were generated with Origin 6.0 to determine LC_x (lethal concentration) values. Avoidance % was calculated as follows: A = (animal number in control / lower BR concentration / first soil) − (animal number in treated soil / higher BR concentration / second soil) ⁄ total number of animals * 100. The chosen % was calculated as follows: C = (number of animals at one side of the Petri-dish) ⁄ 8 * 100. The habitat function of soils is considered to be limited if on average ≥ 75 % of worms (chosen % ≥ 75 %, avoidance % ≥ 50 %) (based on the Poisson-distribution) were found in the control or the treated soil [40].

3 Results and discussions

3.1 pH and element content of BR treated soils

BR addition raised significantly the pH of the whole soils, soil suspensions and extracts starting from 1 % BR amount (except for NH-E, OB-WS and OB-E) (Table 3).

The pH exceeded the threshold value of 9 [42, 43] starting from 10 % BR amount in NH-WS, NH-S, NY-WS and NY-S, and from 5 % BR amount in OB-WS and OB-S.

Table 3 pH in BR treated soils (WS), suspensions (S) and extracts (E), bold: pH > 9. Correlation coefficient (r) between pH and BR amount in soil, bold r > |0.85|

Soil type	C	1 %	5 %	10 %	25 %	50 %	r
NH - WS	8.0 ± 0.0^a	8.1 ± 0.0^b	8.6 ± 0.0^c	**9.1 ± 0.0^d**	**9.5 ± 0.1^e**	**10.3 ± 0.6^f**	**0.90**
NH - S	8.0 ± 0.1^a	8.3 ± 0.0^b	8.7 ± 0.0^c	**9.1 ± 0.0^d**	**9.8 ± 0.0^e**	**10.3 ± 0.0^f**	**0.99**
NH - E	7.7 ± 0.1^a	7.8 ± 0.0^a	8.0 ± 0.0^a	8.3 ± 0.1^b	8.8 ± 0.2^c	**9.6 ± 0.1^d**	**0.99**
NY - WS	5.0 ± 0.2^a	8.5 ± 0.0^b	8.8 ± 0.1^b	**9.4 ± 0.2^c**	**9.8 ± 0.0^d**	**9.7 ± 0.0^d**	**0.97**
NY - S	5.2 ± 0.0^a	8.4 ± 0.1^b	9.0 ± 0.1^c	**9.5 ± 0.1^d**	**10.0 ± 0.0^e**	**9.8 ± 0.0^f**	**0.99**
NY - E	6.5 ± 0.3^a	7.3 ± 0.0^b	7.2 ± 0.1^b	7.6 ± 0.1^b	9.0 ± 0.6^c	**9.3 ± 0.3^c**	**0.93**
OB - WS	8.5 ± 0.1^a	8.6 ± 0.0^a	**9.1 ± 0.0^b**	**9.4 ± 0.0^c**	**9.8 ± 0.1^d**	**10.0 ± 0.0^d**	**0.97**
OB - S	8.8 ± 0.1^a	9.0 ± 0.0^b	**9.5 ± 0.0^c**	**10.0 ± 0.0^d**	**10.2 ± 0.0^e**	**10.3 ± 0.0^e**	**0.99**
OB - E	7.0 ± 0.4^a	7.3 ± 0.1^a	7.8 ± 0.1^b	7.7 ± 0.2^b	8.8 ± 0.1^c	**9.2 ± 0.2^c**	**0.99**

In E the pH 9 was exceeded only upon 50 % BR addition to all soils. There was a linear correlation between BR concentration and the pH. BR addition increased the soil pH as proved by literature data [21, 22, 44] due to its high NaOH content [16, 17].

The total As content exceeded the Hungarian Limit Value at 10 % BR in soils [43] and V surpassed the risk based Dutch Intervention Value at 50 % BR [45] (Table 4). For Cr the Hungarian Limit Value was reached at 5 % BR in NH, 10 % BR in OB and 25 % BR in NY. BR usually contains these elements at a high concentration [46-52].

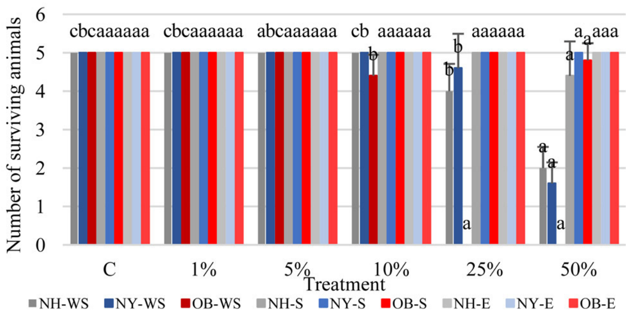

Fig. 1 *E. albidus* lethality in BR treated whole soils (WS), soil suspensions (S) and soil extracts (E)

3.2 Acute mortality tests

The direct contact with whole soils had significant lethal effect on *E. albidus*, while soil suspensions and soil extracts did not cause significant lethality at up to 50 % BR in the soils (Fig. 1).

Table 4 As, Cr and V content of BR treated soils (WS). Bold: amount > Limit Value (As, Cr: [43]; V: [45]); < DL: below Detection Limit; Correlation coefficient (r) between element content and BR amount in soil, bold r > |0.85|

Element	As (mg/kg)	Cr (mg/kg)	V (mg/kg)
Limit	15	75	250
Soil		NH	
C	$10.95 + 0.24^{a}$	$35.23 + 7.86^{a}$	$40.96 + 2.19^{a}$
1 %	$11.74 + 0.58^{a}$	$39.17 + 11.03^{a}$	$65.28 + 3.66^{a}$
5 %	**$18.50 + 1.40^{b}$**	**$80.99 + 16.39^{b}$**	$98.46 + 8.48^{b}$
10 %	**$23.07 + 1.63^{b}$**	**$79.88 + 9.39^{b}$**	$115.03 + 6.89^{b}$
25 %	**$40.73 + 2.43^{c}$**	**$116.73 + 15.50^{c}$**	$189.38 + 5.82^{c}$
50 %	**$69.52 + 8.01^{d}$**	**$176.21 + 6.04^{d}$**	**$342.11 + 31.06^{c}$**
r	**0.98**	**0.93**	**0.99**
Soil		NY	
C	$< DL^{a}$	$19.46 + 12.16^{a}$	$< DL^{a}$
1 %	$4.59 + 0.48^{a}$	$15.09 + 0.00^{a}$	$38.16 + 3.62^{a}$
5 %	$9.45 + 1.55^{a}$	$27.35 + 11.42^{a}$	$66.02 + 9.60^{b}$
10 %	**$15.74 + 3.02^{b}$**	$63.35 + 0.98^{b}$	$116.87 + 8.74^{c}$
25 %	**$33.02 + 3.16^{c}$**	**$107.37 + 16.42^{c}$**	$213.18 + 21.48^{d}$
50 %	**$61.03 + 3.38^{d}$**	**$241.39 + 16.11^{d}$**	**$353.40 + 3.00^{e}$**
r	**0.99**	**0.97**	**0.99**
Soil		OB	
C	$4.8 + 0.00^{a}$	$< DL^{a}$	$33.19 + 0.00^{a}$
1 %	$5.96 + 0.74^{a}$	$4.89 + 8.46^{a}$	$41.22 + 8.70^{a}$
5 %	$9.13 + 1.62^{a}$	$37.69 + 8.66^{a}$	$74.70 + 7.24^{a}$
10 %	**$19.27 + 3.86^{b}$**	**$79.25 + 25.54^{b}$**	$141.77 + 20.35^{b}$
25 %	**$30.10 + 0.87^{c}$**	**$101.11 + 9.44^{c}$**	$198.94 + 11.14^{c}$
50 %	**$59.56 + 3.55^{d}$**	**$199.28 + 24.35^{d}$**	**$343.46 + 53.51^{d}$**
r	**0.98**	**0.97**	**0.98**

The effect of soil type, the sample preparation mode, the BR amount and their interaction influenced statistically significantly the amount of surviving animals. Testing of the whole soil samples instead of extracts is a more sensitive method to measure the toxic effect of BRs on the survival of potworms. This result is supported by previous findings on Direct Toxicity Assessment (DTA), which is of high environmental relevance because it represents all possible interactions between contaminants, ecosystem members and the matrix [53].

According to Sydow et al. [54], in case of testing the toxic effect of metals one should pay more attention not only to the exposition mode (e.g. exposition to free ions), but also to the chosen test soil. The sensitivity of invertebrates was different in artificial OECD and natural soils (e.g. EC_{50}: 3.78–5150 mg Zn/kg in OECD soil and 35.00–7264 mg Zn/kg natural soil) due to the relatively small variability of pore water concentrations of dissolved base cations in OECD soils.

Kuperman et al. [55] also found that the soil physical and chemical properties (e.g. pH, organic matter content or soil particle size) affect the toxicity of pesticides to three species of the genus *Enchytraeus*, including *E. albidus*. Instead of using artificial soils, they suggested to conduct toxicity testing on natural soil types which have properties supporting higher bioavailability of test chemicals.

We found strong significant linear correlation between the number of dead *E. albidus* individuals, BR concentration, pH, total As, Cr and V concentrations in case of the whole soil testing option (Table 5). The pH of WS was always higher than the pH tolerance of *E. albidus* (4.8–7.4 [56]) starting with 1 % BR treatment.

The LC_{20} and LC_{50} were different in the three soils (Table 6). BR in OB was the most toxic for *E. albidus* with LC_{20} of 9 % BR and LC_{50} of 11 % BR, while NY and NH resulted in similar LC values. The animals could tolerate

Table 5 Correlation coefficient (r-value) between the number of dead *E. albidus* individuals in each soil type and sample preparation mode, BR concentration, pH, total As, Cr and V. Italics r > |0.60|. ND: no data

	NH-WS	NH-S	NH-E	NY-WS	NY-S	NY-E	OB-WS	OB-S	OB-E
					r				
BR	*0.80*	0.39	0.00	*0.69*	0.00	0.00	*0.80*	0.39	0.00
pH	*0.74*	0.35	0.00	0.55	0.00	0.00	*0.74*	0.39	0.00
As	*0.74*	ND	ND	*0.66*	ND	ND	*0.84*	ND	ND
Cr	*0.78*	ND	ND	*0.68*	ND	ND	*0.76*	ND	ND
V	*0.79*	ND	ND	*0.66*	ND	ND	*0.79*	ND	ND

Table 6 LC_{20} and LC_{50} values in the whole soil samples

	NH-WS	NY-WS	OB-WS
LC_{20} [%BR]	27.6	33.6	8.9
LC_{50} [%BR]	41.9	44.2	10.7

the highest BR addition (LC_{20} = 33 % BR) in the acidic sandy NY soil, and the population was the most sensitive for BR in the carbonated sandy OB soil.

The literature contains limited number of studies in which the authors examined the effect of this BR on animals. Ujaczki et al. [29] did not detect significant mortality in case of *Folsomia candida* (Collembola), at up to 40 % BR in a sandy soil (pH = 7.5) from the Ajka region in Hungary 1 month after treatment, and at up to 30 % after the 2nd and 8th months of treatment. Based on the complex evaluation of metalloid content, pH and the test results with testorganisms from three trophic levels the authors concluded, that up to 5 % BR can be mixed into the Ajka soil without any mid-term adverse effects on the soil as natural habitat.

In another experiment with the NY soil similar recommendation was given by Ujaczki et al. [22]. In addition, 10 % BR dose in NY, 10 months after BR treatment, resulted in 20 % inhibition for the protozoon *Tetrahymena pyriformis*. Rékási et al. [28] reported mortality of *F. candida* in a 10 cm BR layer covered soil column (soil from Ajka region) also in the deeper soil layers (30–50 cm), but the highest mortality was 13 %.

Some studies examined the individual effect of metals on potworms. Lock and Janssen [57] suggested that terrestrial ecotoxicity data of metals, such as Cd and Zn to *E. albidus*, should rather be correlated with the soil pH and cation exchange capacity than clay and organic matter content. They measured 14d LC_{50} between 83.0–1140 mg Zn /kg dry soil and 55.2–704 mg Cd /kg dry soil in various soil types. The toxic effect of Cd and Zn may be due to disturbance in the cellular redox status of *E. albidus*. Cd and Zn induced significant changes in the antioxidant enzyme activities and substrate levels as well as increased lipid peroxidation, indicating oxidative damage [58]. The form of metal added into the artificial soil has also influenced the results of acute and chronic toxicity tests. E.g. the acute toxicity of $ZnCl_2$ was higher for *E. albidus* than of Zn powder and ZnO, while chronic toxicity was similar [59].

For copper, in case of the acute toxicity tests (21 days) the LC_{50} values were higher than 320 mg $CuCl_2.2H_2O$ /kg dry weight in the three different soil types. However, the juveniles were more sensitive with an LC_{50} value from 97 to > 320 mg $CuCl_2.2H_2O$ /kg dry weight [60]. In case of Zn, Cd, Cu and Pb in a 21 days acute toxicity test the LC_{50} was 610 mg Zn /kg dry weight, 554 mg Cd /kg dry weight, 671 mg Cu /kg dry weight; 4530 mg Pb /kg dry weight in OECD soils, respectively [58].

However, the EC_{50} values in the reproduction test (42 days) were 211 mg Zn /kg dry soil, 58 mg Cd /kg dry soil, 337 mg Cu /kg dry soil, 394 mg Pb /kg dry soil, respectively [61]. EC_{50} values in reproduction tests with *E. albidus* were EC_{50}=637 mg Cr/kg dry soil for chromium (III) [62], and for nickel in OECD soil EC_{50} was 275 mg Ni/kg dry soil, while the 21d LC_{50} was 510 mg/kg dry soil, respectively [63].

Cu and Ni have similar toxicity mechanisms in *E. albidus* which are mainly related to oxidative stress and response to ROS. Additionally, Ni affects immune response pathways [64].

3.3 Avoidance tests

As a pre-screening test we tested the avoidance behavior of *E. albidus* in control vs. control in case of the three different soils (Table 7). The results proved that the potworms do not make a choice between the two sides of the Petri-dish containing the same soils.

E. albidus significantly preferred 1 % BR treated NY soil compared to the control (Fig. 2; Table 8). The 5 %

Table 7 Control-Control avoidance of *E. albidus* in case of different soils

	NH - C		NY - C		OB - C	
	side 1	side 2	side 1	side 2	side 1	side 2
	4	4	5	3	4	4
	5	3	3	5	4	4
	3	5	4	4	4	4
Av. number	**4.00**	**4.00**	**4.00**	**4.00**	**4.00**	**4.00**
St. deviation	1.00	1.00	1.00	1.00	0.00	0.00
Avoidance %	0.00		0.00		0.00	

Table 8 The average number of animals at each side of the Petri-dish and avoidance % (compared to the control or the lower BR concentration). Bold: avoidance % ≥ 50 %.

Pairs		C-BR1 %				
Soil	NH		NY		OB	
Treatment	C	1 %	C	1 %	C	1 %
Number	3.33	4.67	0.17	7.83	4.80	3.20
Avoidance %	-16.67		**-95.83**		20.00	
Pairs		C-5 %				
Soil	NH		NY		OB	
Treatment	C	5 %	C	5 %	C	BR5 %
Number	3.50	4.50	0.50	7.50	2.40	5.60
Avoidance %	-12.50		**-87.50**		-40.00	
Pairs		C-10 %				
Soil	NH		NY		OB	
Treatment	C	10 %	C	10 %	C	10 %
Number	4.83	3.17	4.33	3.67	7.60	0.40
Avoidance %	20.83		8.33		**90.00**	
Pairs		C-25 %				
Soil	NH		NY		OB	
Treatment	C	25 %	C	25 %	C	25 %
Number	6.00	2.00	5.67	2.33	6.67	1.33
Avoidance %	**50.00**		41.67		**66.67**	
Pairs		1-5 %				
Soil	NH		NY		OB	
Treatment	1 %	5 %	1 %	5 %	1 %	5 %
Number	4.17	3.83	4.50	3.50	3.73	4.27
Avoidance %	4.17		12.50		-6.67	

Pairs		BR1-BR10 %				
Soil	NH		NY		OB	
Treatment	1 %	10 %	1 %	10 %	1 %	10 %
Number	5.17	2.83	5.83	2.17	4.80	3.20
Avoidance %	29.17		45.83		20.00	
Pairs		BR1-BR25 %				
Soil	NH		NY		OB	
Treatment	1 %	25 %	1 %	25 %	1 %	25 %
Number	6.00	2.00	6.33	1.67	6.00	2.00
Avoidance %	**50.00**		**58.33**		**50.00**	
Pairs		BR5-BR10 %				
Soil	NH		NY		OB	
Treatment	5 %	10 %	5 %	10 %	5 %	10 %
Number	5.00	3.00	5.50	2.50	6.27	1.73
Avoidance %	25.00		37.50		**56.67**	
Pairs		BR5-BR25 %				
Soil	NH		NY		OB	
Treatment	5 %	25 %	5 %	25 %	5 %	25 %
Number	5.83	2.17	7.17	0.83	5.20	2.80
Avoidance %	45.83		**79.17**		30.00	
Pairs		BR10-BR25 %				
Soil	NH		NY		OB	
Treatment	10 %	25 %	10 %	25 %	10 %	25 %
Number	6.00	2.00	6.50	1.50	5.87	2.13
Avoidance %	**50.00**		**62.50**		46.67	

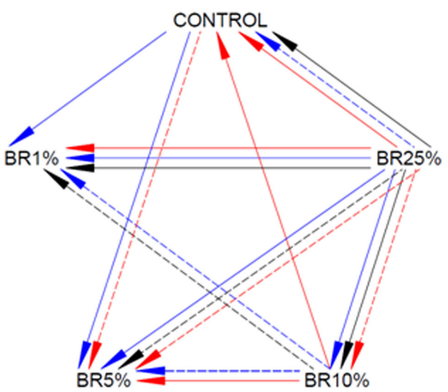

Fig. 2 Choice of *E. albidus* in BR amended soils.
(Black: NH; blue NY; red OB; continuous line: significant ($p < 0.05$) and ≥ 75 % chosen %; interrupted line: significant ($p < 0.05$))

BR concentration was preferred by potworms in case of NY and OB compared to the control. The animals did not make a choice between 1 % and 5 % BR in soils.

Instead of 10 % BR, animals chose C (OB), 1 % BR (NY) and 5 % BR (NY, OB) in the soil. 25 % BR amount was avoided in all soils when compared to the control and the lower BR concentrations. The results show that animals could make a difference between the 10 % and 25 % BR concentration: although both were avoided compared to control, they chose always the lower BR amount in this case. The effect of soil type was not significant for the amount of animals at the two sides of the Petri-dish, but the different pairs and the BR concentration in the soil had a significant effect.

The animals chose the NH soil against NY and OB with ≥ 75 % preference (Fig. 3). OB was selected by animals when paired with NY (except for 1 % and 25 % BR in soil). The effect of soil type and soil pairs was significant for the number of animals at the two sides of the Petri-dish.

The avoidance (or area selection) tests were more sensitive than acute toxicity tests to assess soil, as habitat for potworms (or other terrestrial biota), because in this test the animals reacted to lower concentrations of the tested material (BR) than in the mortality test. Avoidance behavior is an ecologically relevant endpoint, which is

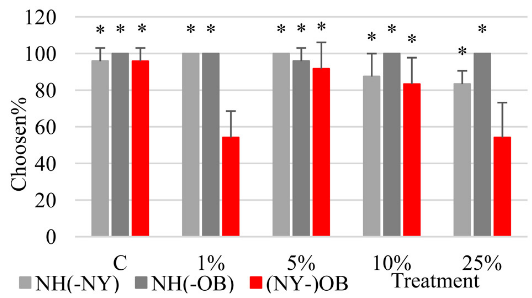

Fig. 3 Choice of *E. albidus* in various soil types containing different BR concentrations. The columns represent the chosen % of the soil type compared to the soil type in brackets. Starred: significant ($p < 0.05$) and ≥ 75 % chosen %

well-useable in soil ecotoxicology tests with Annelids [40, 65, 66]. When the behavior or tolerance of potworms was examined, it was typical that the sublethal end-points (bioaccumulation, reproduction avoidance) were more sensitive compared to lethality [12, 40, 67].

Although the classic avoidance tests, pairing treated samples with the control, are able to forecast the effect of contaminants on potworms, the experimental matrix applied in our study comparing treated samples with each other could provide more detailed information on the behavior of potworms, enabling assessment of the real effects of different soil amendments.

In a metal polluted soil from a mining area *E. crypticus* adult survival was not affected in general by the soil pollution level, but Enchytraeid reproduction was negatively affected, and this was more pronounced over generations [68]. Avoidance behavior can be similarly sensitive to reproduction in the case of testing waste materials. E.g. in case of incineration ash (INC) EC_{50} for reproduction was 16.1 % in control soil and 13.7 % for avoidance. Similarly, EC_{50} for reproduction was 4.1 % in case of contaminated wood chips (WOO) in control soil and 2.9 % for avoidance. *E. albidus* was more sensitive to INC in avoidance tests (EC_{50} = 4.4 % INC in control) than *E. fetida* (EC_{50} < 12.5 %) and *E. crypticus* (EC_{50} = 14.3 %), but less sensitive to WOO (*E. albidus*: EC_{50} = 9.6 % WOO in control, *E. fetida* EC_{50} < 4.5 %, *E. crypticus* EC_{50} = 9.2 %) [69].

In other experiments, the authors focused on the effect of different metals on avoidance of potworms. An avoidance reaction of *E. albidus* of less than 80 % was detected in case of Zn and Cd at up to 180 mg/kg dry LUFA 2.2 soil and 560 mg/kg dry soil, respectively, but > 80 % avoidance was measured for Cu at ≥ 320 mg/kg dry soil. The EC_{50} for avoidance was lower for Zn (EC_{50} = 92 ppm), Cd (EC_{50} = 362 ppm) and Cu (EC_{50} = 133 ppm) compared to LC_{50} from literature. When compared with reproduction EC_{50} values, the difference in sensitivity depended on the type of metal [40]. AC_{50} (the concentration inducing an avoidance of 50 %) for *E. albidus* was 571 mg/kg dry soil for Cd and 215 mg/kg dry soil for Zn in LUFA 2.2 soil upon simultaneous occurrence of Zn and Cd in the soil. These AC_{50} values were higher compared to when these metals occurred individually in the soil [11]. These findings underline the importance of direct toxicity testing of waste materials, which are always the mixture of chemicals.

4 Conclusions

Based on our results ≤ 5 % BR concentration does not have any presumable negative effect on the *Enchytraeus albidus* decomposer soil biota. This finding is in accordance with previous studies of Ujaczki et al. [22, 29]. LC_{20} values from the acute toxicity tests indicated, that BR addition is acceptable for potworms at 9 % in the carbonated sandy soil, at 28 % in the silty soil, and at 34 % in the acidic sandy soil. Direct contact testing of whole soil samples was a more sensitive method, than testing of the soil suspension or soil extract, which indicated no significant mortality up to 50 % BR in soils. This suggests that testing whole soil samples is a better choice for the assessment of the real environmental risks.

Furthermore, the avoidance test results proved to be more sensitive to test the effect of BR on potworms. The acute toxicity (mortality) tests showed that 1–5 % BR treated soils may result in a better habitat for enchytraeids, than the untreated control soil, as potworms chose the BR treated soils over the control. The avoidance tests enabled the assessment of both the negative and positive effects of this soil amendment.

In case of other test organisms there was no significant difference between the acute toxic effect of 10 % and 25 % BR in the acidic sandy soil, but the avoidance test showed clearly that *E. albidus* chose 10 % BR instead of 25 % BR when these concentrations were paired with each other. Compared to the conventional pairing of control and treated samples in the avoidance tests, the pairing of the different treated samples with each other proved to be a more sensitive method to assess preference behavior, so this method could provide us additional information in forecasting the effects of different soil amendments on potworms.

The ecotoxicity test results with *E. albidus* showed that bauxite residue at low concentrations (≤ 5 %) may be used as soil amendment as it provides a better habitat for these organisms.

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