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Acute Ecotoxicological Effects of Bauxite Residue Addition on Mortality and Motion-frequency of *Dendrobaena veneta* and *Enchytraeus albidus* (Annelida) in Three Types of Soils

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Abstract
The bauxite residue is produced in high amounts all over the world. This industrial waste is a possible soil-amendment material. Although the material has been produced in high amounts, it is not frequently reused. We investigated its ecotoxicological effects on two annelid species: *Dendrobaena veneta* and *Enchytraeus albidus*. Two forms of bauxite residue (BR: S – untreated; G – dried, filter pressed, and gypsum neutralized) and three natural soils (NH: Nagyhörcsök, NY: Nyírlugos, OB: Őrbottyán) were examined. To determine the safe concentration of bauxite residue in soil on the short, acute mortality and sublethal behavioral tests (peristaltic motion-frequency) were performed. The bauxite residue addition (<5/10%) raised the pH and water holding capacity level of soils. Both types of the bauxite residue increased the motion-frequency of the worms. The untreated type had an acute mortality effect (>25%). Both species refused the soils containing both bauxite residue types at higher concentrations (>10%). Slight bauxite residue addition may improve the life circumstances of annelids in acidic sandy soils because of the potential rise of the pH level and water holding capacity.

Keywords
bauxite residue, soil-ecotoxicology, Annelida, motion-frequency, mortality

1 Introduction

The bauxite residue (red mud) is an industrial waste of alumina production. Production of each ton of alumina may result 0.7–2 tons of bauxite residue. [1]. This material is alkaline and highly saline [2]. The bauxite residue is a widely used material, e.g. in chemical processes, construction-, environmental technologies [3–5]. Moreover, the bauxite residue is a suitable soil amendment material [5–7] and immobilizes different metals [8–10]. Although 140 million tons [11] were produced year and storage involves potential risks, less than 4 million tons are utilized [5].

The annelids are important decompositor groups. They can affect the soil physical and chemical parameters [12, 13] and increase the plant production [14]. Both the earthworms and Enchytraeidae (potworms) are recommended ecotoxicological test organisms. The systematically examined endpoints are the mortality, reproduction and avoidance (or preference) [15, 16]. The following guidelines prescribe the method of determining the above-mentioned endpoints in environmental risk assessment processes [17–24].

Only a few studies focused on the potential effects of bauxite residue on annelids. Maddock et al. [25] tested how the Metal-Loaded Bauxol Reagent (MLBR), produced from bauxite residue, affected the mortality, weight gain and metal uptake of earthworm (*Eisenia fetida*). The MLBR did not cause mortality in the test population. In higher concentrations (60; 80%) the animals lost weight. Courtney et al. [26] found that the *Allolobophora chlorotica* species did not tolerate the effects of untreated bauxite residue none of them survived it. The test population survived the organically or/and gypsum treated samples but

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lost weight during the test. The A. longa species tolerated only control soil, and bauxite residue from the 12-year-old field restored site. Di Carlo et al. [27] found that the fresh unrehabilitated bauxite residue had mortal effect on *Eisenia fetida* (LC50:37±3.6%). When the bauxite residue addition was more than 25%, it (inhibition >76%). Finngean et al. [28] applied the *Eisenia fetida* avoidance tests. They found that most of the total test population (54%) selected the younger field site which contained 25% gypsum treated bauxite residue.

Kerekes and Feigl [29] found that, the *Enchytraeus albidus* species chose a bauxite residue treated soil (1; 5%) in a soil preference test, when the concentrations did not exceed more than 10%. Although the bauxite residue has been produced in high amounts and it has more options for technological utilization, there is just scarce information about the effects concerning annelids in natural soils. We would like to investigate how the bauxite residue affect different physical-chemical parameters (such as pH, WHC – Water Holding Capacity, metal content) of natural soils. We aimed to determine the safe concentration of bauxite residue for annelids in different natural soils related. In order to answer this question, we added two types of bauxite residue to three different natural soils. The mixed samples were examined with acute lethal and behavioral (peristaltic-motion-frequency) tests by two species (*Dendrobaena veneta*, *Enchytraeus albidus*). The motion-frequency as a pre-screening endpoint provides information about the energy consumption of the organisms. The energy consumption has an indirect effect on the survival of the population. Moreover, this endpoint offers data about the active mitigation behavior.

2 Material and methods

2.1 Materials and experimental set-up

Two different types of bauxite residue (BR) were collected in Hungary (Ajka; 47°5'18.46" N, 17°32'52.09" E) [30]. The first one was untreated and stored in a deposit (S; pH = 10.4±0.1) sampled in 2016. The second one was collected as its counterpart but the material was filter pressed [31] and neutralized with gypsum (2%) (G; pH = 9.4±0.0). To investigate the effects of bauxite residue as soil amendment material, the bauxite residue types were mixed with three different types of Hungarian soils (Table 1 [32–34]). The acidic sandy soil (NY: pH = 4.9) originated from Nyírlugos. The calcareous sandy soil (OB: pH = 7.7) was collected from Őrbottyán. The silty soil (NH: pH = 7.6) was originated from Nagyhörcsök.

Table 1 Soil properties

Properties	NH	NY	OB
Sand:Silt:Clay (%)[a]	17:60:23	85:10:5	81:13:6
WHC (%)[b]	36	30	32
Humus (%)[c]	3.1	0.5	1.0
$CaCO_3$ (%)[c]	1.8	0.0	3.3
pH (H_2O)[c]	7.6	4.9	7.7

[a] Texture [32], [b] Water Holding Capacity [33], [c] from [34],
(NH: Nagyhörcsök, NY: Nyírlugos, OB: Őrbottyán)

The two bauxite residue types were mixed to the soils in various concentrations (w/w%) (Table 2). We tested the physical-chemical parameters (pH, WHC – Water Holding Capacity, XRF – X-ray fluorescence spectroscopy for "total element" content) and the ecotoxicological effects.

The untreated natural soils were used as a control. As a pre-trial experiment, we made the following test (Sections 2.2; 2.4 and 2.5) with undiluted bauxite residue samples (S; G) but just with one control (NH). After these pre-trial experiments, we tested the mixed samples (Table 2). Dried, mixed and sieved (<2 mm) forms of the samples were used for testing.

2.2 Characterization of soil properties

The pH was measured in 1:2.5 soil: distilled water suspension after 30 minutes shaking at 160 rpm [34]. The pH level was typified by classes according to USDA (Table 3 [35]). The total element content was measured by XRF method

Table 2 Tests applied and concentrations [w/w%] to different bauxite residue samples. (100): undiluted bauxite residue sample; []: excluded from the statistical analysis; C: control, WHC: water holding capacity, XRF: X-ray fluorescence spectroscopy

Examined endpoints	Stored bauxite residue	Gypsum treated bauxite residue
pH	C; 1; 5; 7.5; 10; 25; 50; (100)	C; 2.5; 5; 10; 25; 50; (100)
WHC	C; 2.5; 5; 7.5; 10; 25; 50; (100)	C; 2.5; 5; 10; 25; 50; (100)
XRF	C; 5; 10; 25; 50; (100)	C; 5; 10; 25; 50; (100)
Motion-frequency	C; [5]; 10; 25; 50; [100]	C; [5]; 10; 25; 50; [100]
Mortality	C; 1; 5; 10; 25; 50 (100)	C; 10; 25; 50 (100)

Table 3 Class term of pH level in case of soils [35]

Class term	pH range	Class term	pH range
Ultra-acid	<3.5	Neutral	6.6–7.3
Extremely-acid	3.5–4.4	Slightly alkaline	7.4–7.8
Very-strongly-acid	4.5–5.0	Moderately alkaline	7.9–8.4
Strongly-acid	5.6–6.0	Strongly alkaline	8.5–9.0
Slightly-acid	6.1–6.5	Very strongly alkaline	>9.0

with NITON XL3t 600. The WHC was measured by Schinner's [33] method. All the tests were made in three independent replicates at the same time.

2.3 Test organisms

The *Enchytraeus albidus* (described the species in 1837 by Henle) originated from the Budapest University of Technology and Economics, Faculty of Chemical Technology and Biotechnology stock culture. Before each testing, a mixed base culture was prepared from the used stock boxes which were stored on 20±2 °C. Only adult (had clitellum) animals with appropriate length (1–1.5 cm) were tested.

The *Dendrobaena veneta* (described the species in 1886 by Rosa) organisms were bought from special shops. The organisms starved for 2 days in a mixed base-culture (boxes covered by water saturated filter paper) before the tests. During the starving period, the organic material amount was eliminated from the digestion system, so it was not able to work as buffer material. The boxes were stored protected from direct light at constant temperature (20±2 °C). We selected the adult worms to be tested which were long enough (5–8 cm).

2.4 Acute mortality tests

In case of *E. albidus*, the acute mortality test was performed according to OECD 207 [22] modified for enchytraeids by OECD 220 [23]. Shortly, we measured 20 g soils and watered them to 60% of WHC. We placed 5 animals in each vessel (as replicates; glass "jar": V = 360 ml; D = 6 cm). These were stored at constant temperature (20±2 °C), protected from light. After the exposition time (96 h), the number of refused, died or totally immobilized animals (those did not react on tactile stimulus) were counted. An animal was considered "refused", when they were alive and remained in the top layer of soil instead of burrowing itself into the lower layers.

In case of *D. veneta* the OECD 207 [22] guide was followed, but we reduced the mass of the soil sample to 120 g (according to Loureiro et al.'s [36] method) and used a double soil volume. The volume was 40 g/animal dry weight of soil. The test vessels were stored protected from direct light at constant temperature (20±2 °C). The refused (did not burrow layer), died or totally immobilized animals were counted after 7 and 14 days. Three, independent replicates were examined.

2.5 Motion frequency endpoint

We applied a new endpoint, the motion-frequency (behavior) test. This test was supplemented the mortality test in order to gain additional information. This test was made before the acute mortality test on the same test organisms in order to avoid further disturbance caused by their placement. This endpoint was examined in an extra observation time during the mortality test process in the same test vessels.

We defined "motion frequency" as the number of moves in a time unit. The peristaltic motion includes one body part length changing from the shortest to the longest state of body parts. As it is a subjective displacement, one observer performed all the tests to reduce this part of the uncertainty.

Having put one animal in the vessel, we waited the normalization time. The normalization time always took 110-seconds, and it ended when the animals started to move in a well-definable line at almost constant speed. If the normalization time of each animal was more than 10 seconds, we excluded the animal from the test series.

The motions of those animals were counted for 10 seconds which ones had an acceptable normalization time. Before examining a new organism, we waited that the previous one burrowed for lower layer to avoid influencing each other. We examined 3 or 4 animals (depended on the number of excluded animals) in each test vessel. Three, independent replicates were examined.

2.6 Statistical and mathematical analyses

TIBCO Statistica 13.4. software was used to perform the statistical analysis. The significance level (α) was set as 0.05 in all analyses. Abbreviations both in the table and in the models: BR = type of bauxite residue (two levels: S, G); Soil= type of soil (three levels: NY, NH, OB); Conc = concentration of the examined bauxite residue (levels are given in Table 2) in the soil.

Soil properties: Two-sample t-test was used to compare the two undiluted bauxite residue samples (100%) in the case of pH and WHC. Three-way analysis of variance (ANOVA) was used to decide which factors affected the WHC concerning other concentrations (2.5–50%). The fitted ANOVA model contained three fixed factors (Table 2) in crossed design. Due to the absence of replications, only the main effects (BR, Soil, Conc) were evaluated.

Mortality tests: The mortality was analysed by 2 × 2 frequency tables in case of undiluted bauxite residue samples (100%). Other method was applied by other concentrations

(2.5–50%). The statistically sound way to evaluate the mortality as dependent variable would have been the logistic regression. Since the occurrences of non-event (zero) in more factor-combinations, it caused numerical difficulties in estimating parameters of the logistic regression model [37]. A rescue was to use ANOVA for the transformed mortality values. Angular transformation (arcus sinus of square root) function was used to have an approximately normally distributed dataset of constant variance. First, three fixed factors (BR, Soil, Conc), their interactions (BR*Soil, BR*Conc, Soil*Conc, BR*Soil*Conc) and the random effect of the reactor were evaluated. The latter was nested in the third-order interaction dictated by the experimental design. The effect of the reactor was not significant in this case either.

Motion-frequency test: The motion-frequency is a Poisson distributed random variable (count data), thus this dataset was evaluated with Poisson regression (GLZ: Generalized Linear Model) separately for the two examined test species. In case of undiluted samples (100%) the silty soil (NH) was the chosen reference level in the Poisson regression model. The only factor was the medium related to soil or bauxite residue types. In case of other concentrations (2.5–50%), the fitted model contained the three effects and their interactions in case of investigating the effect of bauxite residue addition (as Section 2.2). A random factor, i.e. the test vessels was added to both models, as well. The levels of the vessels could be interpreted only within one combination of the other three factors which means that it was nested in the three-way interaction. The analysis of variance on the square root transformed dependent variable was performed to check if we could get rid of this effect. As the effect of the random factor was not significant, in the final GLZ model only the effects of the crossed structure of the other three factors were evaluated with Type III likelihood-ratio test. The assumptions of the model were checked and accepted. For analysis, it should have been noted that the Likelihood Ratio (LR) test statistics follows chi-square distribution only in the case of large sample sizes [38].

Moreover, we calculated "Stimulating %" to compare the two test species, which is a derived value, as well. This method was used in case of the undiluted bauxite residue samples (100%) and other concentrations (2.5–50%) too. We used the pieces of moving (as Poisson data) by the calculation. This value facilitated the comparison of relative changes concerning test species. The formula was the following:

$$\text{Stimulating\%} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times (-100).$$

3 Results

3.1 Soil properties

The treatment of bauxite residue could influence the properties. Both bauxite residue types (100%) had significantly higher pH and WHC levels than the reference soil (NH). In case of undiluted bauxite residue samples, the treatment of bauxite residue by gypsum reduced significantly both the pH level (S:10.4±0.1; G:9.4±0.0) and water holding capacity (S:51.8±3.4; G:33.4±1.0). It did not change severally the composition of elements (Table 4).

The NY soil had the lowest pH level. The other two soils (OB, NH) were similar to each other. Although addition of bauxite residue increased the pH of soils, the rates were not the same. The bauxite residue addition had positive effects just in case of acidic soil (NY) because of the originally lower pH level. The untreated bauxite residue (S) caused greater rise in pH level than the gypsum treated (G) one. Only 1% untreated bauxite residue (S) addition raised the pH in the neutral class (Table 3). In case of treated one (G) maximum 5% addition affected beneficially to rose the pH level to the neutral zone (Fig. 1).

The highest safe concentrations depended on the soil type (the highest safe examined concentrations: NH-G:C; NH-S:C; NY-G:10%; NY-S:5%; OB-G:5%; OB-S:5%).

Table 4 Total element concentrations by X-ray fluorescence spectroscopy (G: gypsum treated, S: stored bauxite residue)

Element	G	S
As	144.7±8.1	152.1±2.7
Co	83287.5±8870.4	111328.5±3113.1
Cr	436.4±40.3	349.8±15.0
Cu	106.3±15.4	126.1±3.1
Hg	943.5±279.4	1063.4±216.3
Mo	7.5±.06	17.1±1.6
Ni	402.8±15.8	365.9±33.1
U	25.2±2.1	27.1±4.8
Zn	83.7±2.2	100.7±7.2
V	530.3±103.0	709.2±33.4

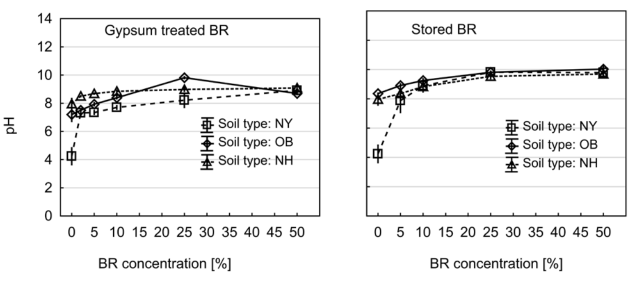

Fig. 1 Dependence of pH level on BR concentrations for different types of soil and bauxite residue types (means with 95% confidence intervals) (BR: bauxite residue, NH: Nagyhörcsök, NY: Nyírlugos, OB: Őrbottyán)

Typically, 5% or 10% bauxite residue addition increased metal concentration of the soil (As, Cr, V) above the threshold value. Concerning the three metals together the highest safe concentration depended on the original metal contamination of control soil (Table 5, Appendix A [39, 40]).

The three main effects were significant for water holding capacity (Table 6, Appendix B). The interaction effects may not be tested. The water holding capacity was more increased by untreated bauxite residue (S) than by the treated one (G). Although almost all the concentrations of bauxite residue increased the WHC level, this effect was statistically significant only in the higher concentrations (>10%). The water holding capacity improving effect was higher in case of sandy soils (NY, OB) with originally lower WHC.

3.2 Mortality tests of two test-species

Both undiluted bauxite residues (100%) caused severe mortality to *Dendrobaena veneta* test species. The average survival (after 14 days) was G:22.2±38.5%, S:55.6±50.9%, but the difference between the bauxite residue type was not significant. In case of *Enchytraeus albidus* test species, the average surviving was G:93.4±11.5%, S:0±0.0% and the difference between the two types of bauxite residue (100%) was significant. All the survived animals refused both bauxite residue types (100%) in case of both test species.

The *D. veneta* test-species survived all the examined concentrations. However the animals totally refused the ≤10% (S) or ≤25% (G) concentrations. The *E. albidus* survived almost all of the examined concentrations of gypsum treated bauxite residue (G) in each soil. Similarly, the *D. veneta* test species, the *E. albidus* totally refused the 10% or higher concentrations of this bauxite residue (G) in case of all types of soils. Only the untreated bauxite residue (S) caused meaningful mortality (>20%) (Table 7, Fig. 2).

There was no death expected by the model in control soils (C) and at lower concentrations (1–5%) of bauxite residue. In the calcareous sandy soil (OB), the 10% concentration reduced significantly the survival in test population. In case of the other two types of soils (NH, NY), the 25% and 50% concentrations had significant effects. The mortality was the highest at higher (25%, 50%) bauxite residue concentrations in the calcareous sandy soil (OB).

3.3 Motion-frequency tests

The *Enchytraeus albidus* was sensitive to undiluted bauxite residue (100%). Both types of undiluted bauxite residue (G, S) increased significantly the peristaltic motion-frequency of animals compared to the control (NH). The treated sample (G:137.4±0.0%) caused less motion-frequency stimulation than the untreated one (S:141.6±19.7%). The difference between the bauxite residue types was not significant. Examining the other levels of BR concentration, the bauxite residue addition increased the peristaltic motion-frequency of *E. albidus* (Table 8, Fig. 3).

Every investigated effect was statistically significant in both test species. As it mentioned in Section 2.5, the Chi-Square was less reliable, the graph is more informative (Fig. 3).

The parameter estimates and the calculated values give more reasonable information about the effects. The expected motion-frequencies were similar in the two

Table 5 The safe concentration in case of different types of soil related to three examined metals (As, Cr, V). "Safe" was when the amount of metal was not higher than threshold value. (NH: Nagyhörcsök, NY: Nyírlugos, OB: Őrbottyán, G: gypsum treated, S: stored bauxite residue)

	NH		NY		OB	
	G	S	G	S	G	S
As	C	C	10%	5%	5%	5%
Cr	5%	C	10%	10%	10%	5%
V	25%	25%	25%	25%	25%	25%
Safe conc.	C	C	10%	5%	5%	5%

Table 6 ANOVA for the effects of factors on Water Holding Capacity (BR: bauxite residue, Soil: examined soil type, Conc: concentration of bauxite residue)

	Sum of Squares	Degr. of Freedom	*MS*	*F*	*P*
BR	317.89	1	317.89	17.893	0.000
Soil	4491.50	2	2245.75	126.410	0.000
Conc.	1548.68	5	309.74	17.435	0.000
Error	1261.36	71	17.77		

Table 7 The ANOVA table of the transformed mortality and the investigated factors and interactions (BR: bauxite residue, Soil: examined soil type, Conc: concentration of bauxite residue)

	SS	Degr. of freedom	*MS*	*F*	*P*
Intercept	8233.51	1	8233.51	170.2234	0.000
BR	21056.09	1	21056.09	435.3233	0.000
Soil	1874.25	2	937.12	19.3745	0.000
Conc	42168.80	5	8433.76	174.3635	0.000
BR*Soil	6406.39	2	3203.19	66.2243	0.000
BR*Conc	19241.73	3	6413.91	132.6042	0.000
Soil*Conc	10276.59	10	1027.66	21.2463	0.000
BR*Soil*Conc	5161.62	6	860.27	17.7856	0.000
Error	4643.41	96	48.37		

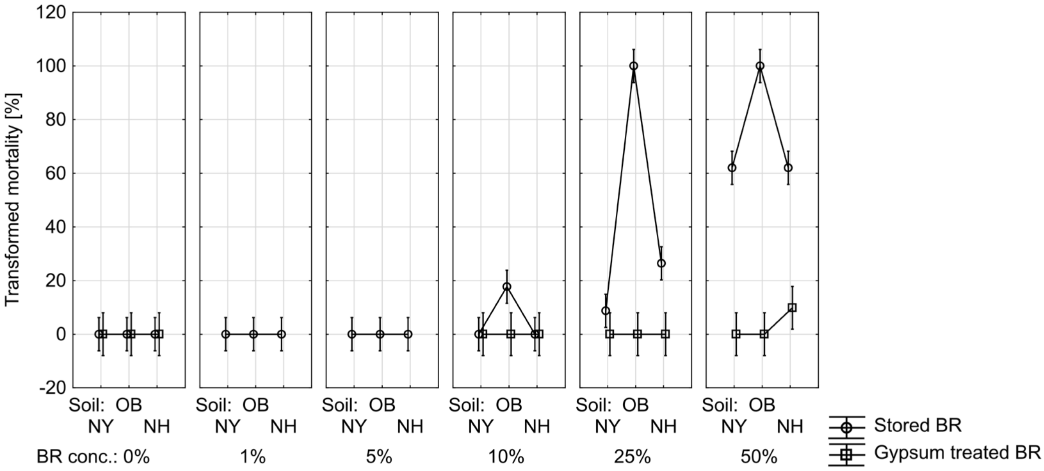

Fig. 2 Transformed mortality of *E. albidus* estimated by the model (with 95% confidence intervals) for different types of soils in the function of added bauxite residue concentration (BR: bauxite residue, NH: Nagyhörcsök, NY: Nyírlugos, OB: Őrbottyán)

Table 8 Type III likelihood-ratio test for the main effects and interactions on motion-frequency of *E. albidus* and *D. veneta* (BR: bauxite residue, Soil: examined soil type, Conc: concentration of bauxite residue)

	Degr. of freedom	Log-Likelihood	Chi-Square	*P*
		Enchytraeus albidus		
BR	1	37533.13	11.3	0.001
Soil	2	37411.88	253.8	0.000
Conc	3	37360.50	356.6	0.000
BR*Soil	2	37507.76	62.1	0.000
BR*Conc	3	37490.97	95.7	0.000
Soil*Conc	6	37503.99	69.6	0.000
BR*Soil*Conc	6	37492.38	92.8	0.000
		Dendrobaena veneta		
BR	1	1966.68	18.7	0.000
Soil	2	1957.51	37.1	0.000
Conc	4	1920.19	111.7	0.000
BR*Soil	2	1961.98	28.2	0.000
BR*Conc	4	1966.91	18.3	0.001
Soil*Conc	8	1947.59	56.9	0.000
BR*Soil*Conc	8	1945.33	61.4	0.000

sandy soils (NY, OB) related to both bauxite residue types (G, S). The untreated bauxite residue addition caused higher changes. The lowest motion-frequency was found in the silty control soil (NH:4.85) which was the reference level. For the treated bauxite residue (G) the motion-frequency was 0.85 times lower than the other (S). If this type (G) was added to the soils, the effect of the treatment depended on the type of the soils i.e. there was an interaction between these two factors. In silty soil (NH) the motion frequency increased significantly only at the 50% dose of BR compared to the control soil (C). In sandy soils (NY, OB) the trend of the increase in motion frequency was very similar to each other but it differed from the silty soil (NH). This tendency was not observable by untreated bauxite residue (S). There is an interaction between the bauxite residue type and concentrations factors. In addition, it could be observed that the interaction between the soil type and conc. depends on the bauxite residue type (three-way interaction). Concerning the untreated bauxite residue (S), if the acidic sandy soil (NY) was used (instead of NH) the motion-frequency was 1.76 times higher (8.55) than in the reference soil. The calcareous sandy soil (OB) generated similar motion-frequency to the other sandy soil, the expected value of the motion- frequency was 8.40. The highest value of motion-frequencies was predicted by the 50% concentration of untreated bauxite residue (S) with sandy soils (NY: 12.00; OB: 12.15). Compared to these soils (NY, OB), the silty soil (NH) resulted only 9.60 motion-frequency value.

The *Dendrobaena veneta* test species was sensitive to undiluted bauxite residue (100%). Both bauxite residue types (G, S) increased significantly the peristaltic motion-frequency of animals compared to the control (NH). The untreated (S:70.8±7.9%) sample caused higher motion-frequency stimulations than the treated one (G:58.3±7.0%). The difference between the bauxite residue types was not significant. Based on LR values the concentration of the added bauxite residue was the most important factor in case of *D. veneta* (Table 8, Fig. 4). The changes of motion-frequency were different in case of different types of soils and/or bauxite residue types (interaction between soil- and bauxite residue types). The control soils (without bauxite residue) showed more similar

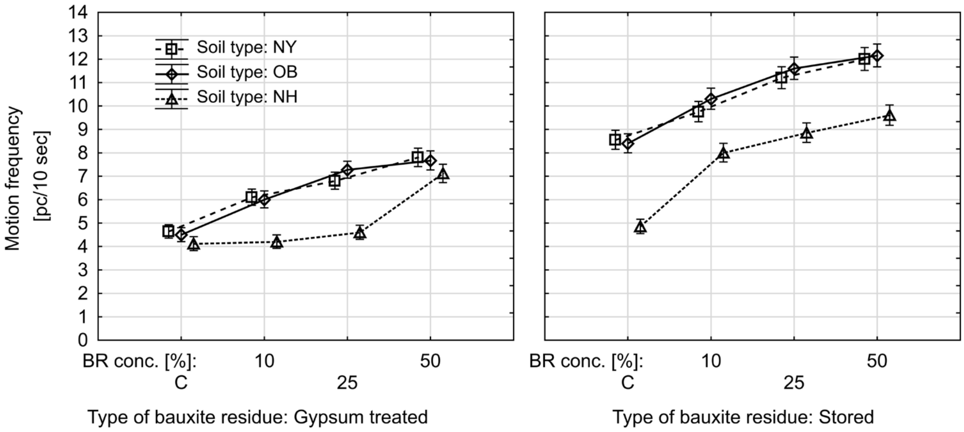

Fig. 3 The LS means plots (with 95% confidence intervals) of the fitted generalized linear model *E. albidus* (BR: bauxite residue, NH: Nagyhörcsök, NY: Nyírlugos, OB: Őrbottyán)

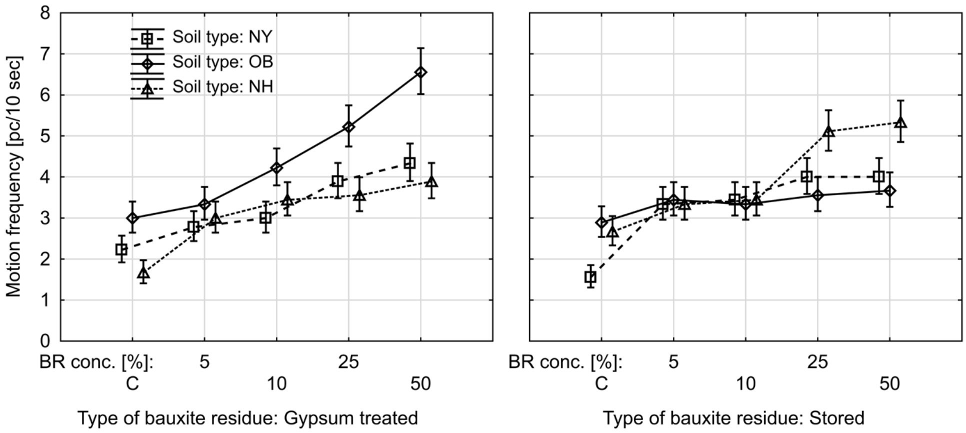

Fig. 4 The LS means plots (with 95% confidence intervals) of the fitted generalized linear model *D. veneta* (BR: bauxite residue, NH: Nagyhörcsök, NY: Nyírlugos, OB: Őrbottyán)

behavior in terms of the motion-frequency than in case of *E. albidus*. The expected value of the motion-frequency of *E. albidus* was approx. 1.7 times higher in the silty (NH) and calcareous sandy (OB) soils than acidic sandy (NY) soil after untreated bauxite residue addition.

Although the motion-frequency was increased by concentration, the effect was influenced by the type of soil and bauxite residue. The test-organism had similar motion-frequency changes at 5% bauxite residue concentration as in gypsum treated bauxite residue (G). In case of untreated bauxite residue (S), these concentrations were 5% and 10% (Fig. 4).

The two test-species reacted differently to bauxite residue addition (Table 9, Appendix C). The motion-frequency changes of *D. veneta* were always lower. The treated bauxite residue (G) caused smaller change in "Stimulating%" than the stored one (S). The differences were the highest for the acidic sandy soil (NY) in case of both species. It seemed that the 10% concentration caused hormesis by *E. albidus*.

4 Discussion

According to our results the addition of bauxite residue in a low concentration (S:5%; G:10%) could improve the environmental circumstances of *Dendrobaena veneta* and *Enchytraeus albidus* species in acidic soil having insufficient water holding capacity.

The improving effects of bauxite residue addition depend on the original properties of soil and bauxite residue [41–44]. The material is able to increase the pH level of soils [6, 7, 45].

The pH increasing might have been positive in case of acidic soils (NY) and it was unpleasant for the other two soils (NH, OB). Regarding the originally neutral or slightly alkaline soil (NH, OB) further pH increase had no ecotoxicological advantages. Moreover, the bauxite residue addition can rise the water holding capacity of soils [7, 46]. Only the higher concentrations (>10%) were affected negatively based on these studies. On the other hand, the bauxite residue usually contains some potentially toxic concentrations of elements and metals [47–49].

Table 9 Average motion-frequency changing (Stimulating%) with factorizing to gypsum treated experimental series controls (G: gypsum treated, S: stored bauxite residue, BRX: bauxite residue with a concentration of X%, NH: Nagyhörcsök, NY: Nyírlugos, OB: Őrbottyán)

	NH	NY	OB
	Dendrobaena veneta		
G-BR5	12.5±2.4	19.1±2.6	11.1±1.1
G-BR10	35.4±0.9	38.1±2.3	40.7±1.9
G-BR25	33.3±1.8	66.7±8.7	70.4±9.6
G-BR50	45.8±2.3	85.7±6.6	118.5±7.0
S-BR5	25.0±2.5	171.4±34.3	20.0±4.0
S-BR10	29.2±1.6	182.1±20.3	16.0±2.7
S-BR25	91.7±3.5	235.7±19.6	24.0±2.6
S-BR50	95.8±7.1	246.4±11.5	28.0±2.5
	Enchytraeus albidus		
G-BR5	147.4±11.1	125.9±33.6	149.1±22.7
G-BR10	2.7±4.7	78.9±5.6	89.5±10.5
G-BR25	12.2±2.3	100.0±8.4	130.7±15.4
G-BR50	73.0±9.4	128.5±5.1	103.5±42.5
S-BR5	36.3±2.1	4.7±0.2	8.5±0.6
S-BR10	55.1±2.3	7.6±0.3	12.2±0.8
S-BR25	69.9±2.7	16.7±0.8	20.5±0.9
S-BR50	83.0±4.5	21.8±1.3	24.1±1.2

We found that the application was safe at lower concentrations (<10%), as it was shown by Ujaczki et al. [7, 50] and Kerekes and Feigl [29]. Due to this aspect, we should pay attention to the original metal contamination of soils before using the bauxite residue to improve different soils. Although the earthworm acute mortality test is recommended for characterizing the ecotoxicological risks of different types of waste [51], we found that the bauxite residue addition had no acute mortality effect on *D. veneta*. This result fits to Maddock et al.'s [25] results. Moreover, it confirms Courtney et al.'s [26] results, who did not find acute toxic effects in case of *Allolobophora chlorotica* and A. longa (especially in case of the untreated fresh sample). The lower concentrations (<10%) of both types of bauxite residue (S, G) were safe in case of both species.

The *E. albidus* was more sensitive to the effect of bauxite residue addition. The higher concentrations (25, 50%) caused significant mortality which result was in accordance with Kerekes and Feigl's [29] result. Although the undiluted treated bauxite residue (G) did not have lethal effect on *D. veneta*, both test species refused the higher (25, 50%) concentrations of both types of bauxite residue (S, G). The bauxite residue addition influenced the behavior of both species as well. The potworms were usually more resistant (except in silty soil (NH)) related to the peristaltic motion-frequency endpoint. The originally lower motion- frequency was detected in case of *D. veneta* test species in untreated soils (C). The motion-frequency was stimulated more compared to *E. albidus* because of the originally lower motion- frequency of *D. veneta*. Our results confirmed the literature data, whereby the motion frequency is different in case of Annelida species with different body size or mass [51–53].

As it was found earlier, the soil type influenced this behavior [54]. Taking into consideration the data, we found that the lowest concentrations (<10%) of bauxite residue were not only safe but could also improve the acidic or sandy soils. Since the soil pH can be a limiting factor on earthworm distribution [55], the pH increasing effect can be positive in acidic soil. Although the *E. albidus* pH optimum range is 6.8–7.0 with 55–65% soil moisture, the animals can tolerate 4.8–7.4 pH [56]. Despite this result, we found that the animals could survive higher pH level (max. ~9 pH) in a short term. It is in higher accordance with Grafe and Schmelz [57] results, that this species tolerated slightly alkaline or acid conditions too.

The *D. veneta* prefers slightly basic pH level and the species has high moisture tolerance (67.4–84.3%) [58]. There is no accurate information about pH tolerance or optimum of *D. veneta*, but generally the earthworm species tolerated wide pH range (e.g. *Eisenia fetida* 5–9 soil pH level) [59].

Due to the pH and water holding capacity increasing features of moderated bauxite residue addition, we can utilize this material to optimize the circumstances in the degradated or acidified soils. This hypothesis was confirmed by the results of Ujaczki et al. [7, 50], Finngean et al. [28] and Kerekes and Feigl [29].

5 Conclusion

The bauxite residue addition could improve the properties of acidic or degradated soils. When the soil pH is neutral or higher, we should pay attention that, the bauxite residue addition is likely to cause further pH level increase. All the concentrations increased the pH level of soils. In case of acidic sandy soil (NY) <2.5% (S) or <5% (G) bauxite residue addition raised the pH level to the neutral class. The addition of lower concentrations (<10%) were safe according to XRF analysis of all tested soil types.

The untreated bauxite residue (S) caused significant mortality to potworms in case of higher concentrations (25; 50%). The addition of gypsum was likely to reduce the potential lethal effect of material on annelids by this type of bauxite residue.

Both types of bauxite residue influenced the behavior of the animals. The increase of motion-frequency was concentration-depended. Both species refused the higher concentrations of bauxite residue (25; 50%). In this research, the bauxite residue was a possible soil-amendment material to acidic soil with inefficient water holding capacity. The treated bauxite residue had lower toxic effects and was safe in higher concentrations. Therefore, the treated bauxite residue seems to be a better soil amendment material. In the future it will be recommended investigating the ecotoxicological and environmental effects of different types of bauxite residue mixed with other acidic or/and sandy soils.

According to our and the literature information, the smaller concentrations of bauxite residue is likely not to have ecotoxicological risks and the material is able to improve a few soil properties. It is necessary to examine the potential long-term effects of its utilization as soil amendment material, because it may be a well-appropriate reuse option. Furthermore, we should collect more information about the short- and long-term sensitivities of other taxonomic groups before the field application. Although we did not find significant risk in case of less concentrations (<10%), the examiners should focus on the sublethal and chronic effects of addition (e.g. avoidance, reproduction) in the future.

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Appendix A

Table 10 As, Cr and V content of BR treated soils. Bold: amount > threshold value (As, Cr: Hung. 6/2009 [39]; V: Swarties, 1999 [40]); <DL: below detection limit, T. H.: Threshold value, S: stored, G: gypsum treated bauxite residue, NH: Nagyhörcsök, NY: Nyírlugos, OB: Őrbottyán

	As [mg/kg]		Cr [mg/kg]		V [mg/kg]	
T.H.	15		75		250	
	S	G	S	G	S	G
			NH			
C	11.0±0.2	11.0±0.2	35.2±7.9	35.2±7.9	41.0±2.2	41.0±2.2
5%	**18.5±1.4**	**17.4±4.1**	**81.0±16.4**	53.8±14.4	98.46±8.48	81.8±31.4
10%	**23.1±1.6**	**23.6±0.9**	**79.9±9.4**	**101.2±4.9**	115.0±6.89	112.19±9.2
25%	**40.7±2.4**	**42.5±5.0**	**116.7±15.5**	**149.7±14.0**	189.4±5.8	183.5±44.3
50%	**69.5±8.0**	**71.6±16.9**	**176.2±6.0**	**262.6±64.9**	**342.1±31.1**	**353.4±83.2**
			NY			
C	<DL	<DL	19.5±12.2	19.5±12.2	<DL	<DL
5%	9.45±1.6	7.2±1.99	27.4±11.4	17.8±5.5	66.0±9.6	36.4±0.0
10%	**15.7±3.0**	14.3±7.0	63.4±1.0	58.9±27.0	116.9±8.8	73.7±47.3
25%	**33.0±3.2**	**34.6±0.7**	**107.4±16.4**	**167.0±30.6**	213.2±21.5	195.8±39.6
50%	**61.0±3.4**	**57.8±22.7**	**241.4±16.1**	**261.4±68.4**	**353.4±3.0**	245.1±18.4
			OB			
C	4.8±0.0	4.8±0.00	<DL	<DL	33.2±0.0	33.2±0.0
5%	9.1±1.6	11.9±4.2	37.7±8.7	33.2±17.9	74.7±7.2	56.3±27.2
10%	**19.3±3.9**	**17.2±3.9**	**79.3±25.6**	70.2±2.2	141.8±20.4	86.3±15.0
25%	**30.1±0.9**	**40.1±3.5**	**101.1±9.4**	**179.8±44.6**	199.0±11.1	235.4±58.9
50%	**59.6±3.6**	**101.1±2.2**	**199.3±24.4**	**321.6±70.7**	**343.46±53.5**	**365.6±67.8**

Appendix B

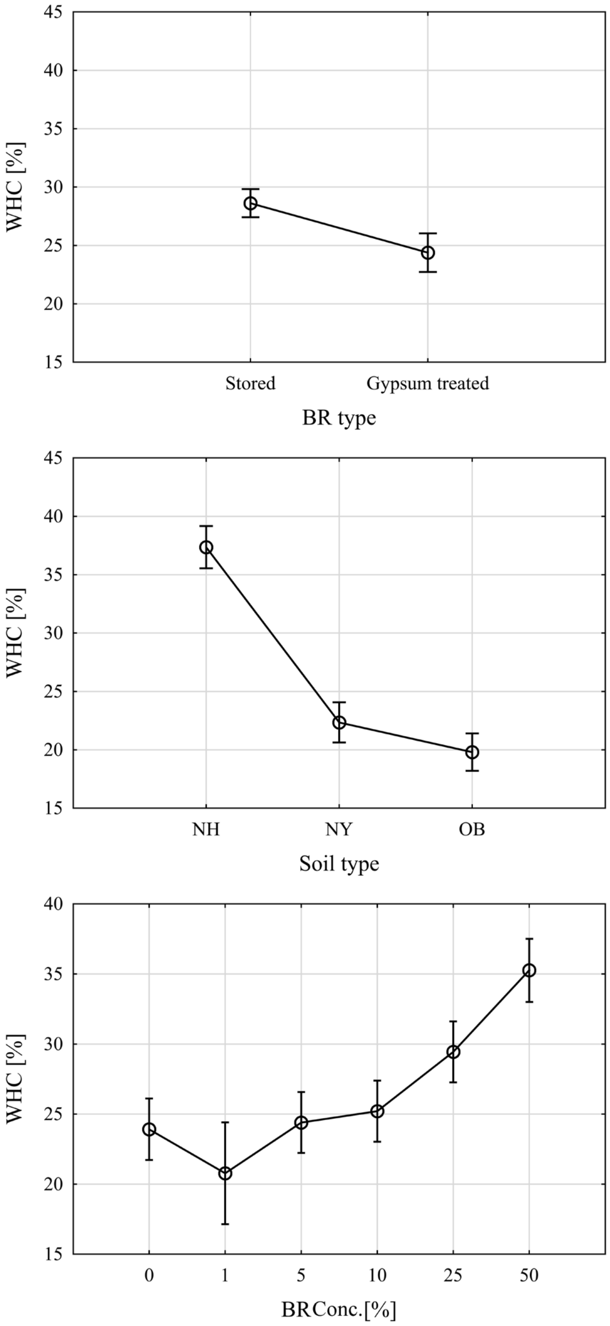

Fig. 5 Weighted means plots of WHC (with 95% confidence intervals) (BR: Bauxite residue, NH: Nagyhörcsök, NY: Nyírlugos, OB: Őrbottyán)

Appendix C

Table 11 Average, original motion-frequency of different test species in different (NH: Nagyhörcsök, NY: Nyírlugos, OB: Őrbottyán)

	Dendrobaena veneta		*Enchytraeus albidus*	
	Gypsum treated	Stored	Gypsum treated	Stored
NY (C)	2.2±0.07	1.6±0.09	4.6±0.20	8.5±0.12
OB (C)	3.0±0.06	2.9±0.07	4.5±0.22	8.4±0.18
NH (C)	1.7±0.09	2.7±0.07	4.1±0.20	4.9±0.25