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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 amount all over the world. This industrial waste is a possible soil-ameliorant material. Although the material has been producing in high amount, it is not frequent to reuse it. 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 in short term, the acute mortality and sublethal behavior 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 [illegible] had an acute mortality effect (> 25 %). Both species refused the higher concentration soils (≥ 10 %) of both types of bauxite residue. [illegible] bauxite residue addition may improve the life circumstances of annelids in acidic sandy soils because of the pH level and water holding capacity potential rise.

Keywords

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

1 Introduction

The bauxite residue (red mud) is an industrial waste, which is produced 0[illegible]2 tons each ton of alumina production [1]. This material is alkaline and highly saline [2]. The bauxite residue is a widely used material e.g. in chemical processes, construction-, environmental addition [3–5]. Moreover, the bauxite residue is a suitable soil amendment material [5–7] [illegible] immobilize different metals [8–10]. Although 140 million tons [11] were produced a year and it has to be stored with 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 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 was significantly impacted survival (> 28 %) and reproduction

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(inhibition > 76 %). Finngean et al. [28] examined the *Eisenia fetida* area choose 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 amount and it has more options for technological utilization, there are just a little information about the effects concerning annelids in natural soils. We would like to investigate how the bauxite residue affect in 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 in different natural soils related to annelids. 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 behavior (peristaltic-motion-frequency) tests by two species (*Dendrobaena veneta*, *Enchytraeus albid*[illegible]). The motion-frequency as pre-screening endpoint prov[illegible]es information about energy consumption of organism[illegible]. The energy consumption has indirect effect on the surviv[illegible]ing of the population. Moreover, this en[illegible]oint [illegible]ers data about the active mitigation behavior[illegible]

2 Material and methods

2.1 Materials and experim[illegible]tal set-up

Two different types of [illegible] residue (BR) w[illegible] collected in Hungary (Ajka; [illegible]5'18.4[illegible]N, 17°32'52.09[illegible]E) [30]. The first one w[illegible] untreated and [illegible]red in a deposit (S; pH = 10.4±0[illegible] in 2016. The second [illegible] was collected as its counte[illegible]art, but t[illegible] material was filter pressed [31] and neu[illegible]ized wi[illegible] gypsum (2 %) (G; pH = 9.4±0.0). To investiga[illegible] effects of [illegible]uxite residue as soil ame[illegible]aterial, [illegible] bauxi[illegible]esidue types were mixed with three d[illegible]erent typ[illegible]ungarian soils (Table 1 [32–34]). The a[illegible]ic sandy soil (NY: pH = 4.9) was originated from N[illegible]ugos. [illegible]careous sandy soil (OB: pH = 7.7) was [illegible]llected from Őrbottyán. The silty soil (NH: pH = 7.6) wa[illegible]riginated from Nagyhörcsök.

Th[illegible]two bauxite residue types were mixed to the soils in various concentrations (w/w%) (Table 2). We tested both more physical-chemical parameters (pH, WHC – Water Holding Capacity, XRF – X-Ray Fluorescence spectroscopy as total element content) and ecotoxicological endpoint.

Table 1 Soil properties a Texture [32], b Water Holding Capacity [33], c Humus, c $CaCO_3$, c pH [34] (NH: Nagyhörcsök, NY: Nyírlugos, OB: Őrbottyán)

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

Table 2 Tests applied and concentra[illegible]s [w/w[illegible]] to different bauxite residue samples. (100): und[illegible]ed bauxi[illegible]ue sample; [] [illegible]cluded from the statistical anal[illegible], C: control, WH[illegible] Water Ho[illegible]g Capacity, XRF[illegible] Fluorescence spect[illegible]

Examined endpoints	Stored bauxite [illegible]idue	Gypsum treated bauxite residue
pH	[illegible]5; 7.5; 10; 25; 50; [illegible])	C; 2.5; 5; 10; 25; 50; (100)
WH[illegible]	C; 2.5; 5; 7.5; 10; 25; 50; (100)	C; 2.5; 5; 10; 25; 50; (100)
X[illegible]	C; 5; 1[illegible]5; 50; (100)	C; 5; 10; 25; 50; (100)
Mo[illegible]n-freq[illegible]cy	C; [5]; 10[illegible]5; 50; [100]	C; [5]; 10; 25; 50; [100]
Mortal[illegible]	C; 1[illegible]; 25; 50 (100)	C; 10; 25; 50 (100)

[illegible]ntreated natural soils were used as control. [illegible]s a pre-trial experiment, we made the following test (Subsections 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 with NITON XL3t 600. The WHC was measured by Schinner's method [33]. All the tests were made in three, independent replicates at the same time.

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

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. We made a mixed base-culture from the used stock boxes before the testing, 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) organism were bought from special shops. The organisms starved for 2 days in a mixed base-culture (boxes covered water saturated filter paper) before the tests. During the starving period the organic material amount eliminated from the digestion system, so it was not able to work as puffer 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 modified for enchytraeids by OECD 220 [23]. Shortly, we measured 20 g soil and watered the 60 % of WHC. We placed 5 animals in each vessel (as replicates; glass "jar": V = [illegible] ml, D = 6 cm). These were stored at constant temperature (20±2 °C), protected from light. After the exposure time ([illegible]), the number of refused, died or totally immobilized animals (those did not react on tactile stimulus) were counted. We called an animal "refused" when they were alive and stayed in the top layer of soil instead of burrowing 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) we used a double of their volume which was 20 g/animal dry weight of soil [4[illegible]animal]). 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 new endpoint, the motion-frequency (behavior) test supplementing the mortality test in order to gain extra information. This test was made before the acute mortality test on the same test organisms in order to avoid the further disturbance caused by the placing. 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 uncertainty.

Having put one animal in the vessel, we waited the normalization time. The normalization time always took 1–10-seconds and it ended when the animal 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 one had an acceptable normalization time. Before examining a new organism, we waited that the previous one burrowed to 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. Significance level (α) was set as 0.05 for 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 3) 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) may have been 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, Soils, Conc), their interactions 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 Subsection 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 a derived value, as well. This method was used both 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 one (NH). The treatment of bauxite residue 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).

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.[illegible]13.1
Cr	436.4±40.3	3[illegible]±15.0
Cu	106.3±15.4	[illegible]1±3.1
Hg	943.5±279.4	1063.[illegible]6.3
Mo	7.5±.06	17.1±1.[illegible]
Ni	402.8±15.8	[illegible]5.9±33.1
U	25.2±2.1	27.1±4.8
Zn	83.[illegible]2	100.7±7.[illegible]
V	[illegible]±103.0	709.2[illegible].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 higher 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 so rose the pH level by neutral zone (Fig. 1).

The NOEC (No Observed Effective 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 %). Typically, 5 % or 10 % bauxite residue addition caused risky metal-concentration. 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) interaction effects may not be tested. The water holding capacity was more increased

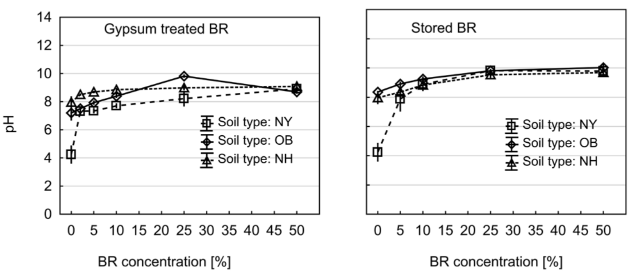

Fig. 1 Dependence of pH level on BR concentrations for different types of soil and bauxite residue types (means with 95 % confidence intervals) (BR: bauxite residue, NH: Nagyhörcsök, NY: Nyírlugos, OB: Őrbottyán)

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 did 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 %
NOEC	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		

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 residue (100 %) caused severe mortality by *Dendrobaena veneta* test species. The average surviving (after 14 days) was G:[illegible]2.2±38.5 %, S:[illegible].6±50.9 %, but the difference between the types of bauxite residue was not significant. In case of *Enchytraeus albidus* test species, the average surviving was G:93.4±11.5 %, S:0±[illegible] % and the difference between the two types of bauxite residue (100 %) was significant. All the survived animals refused both bauxite residue types (100 %) related to both test species.

The *D. veneta* test-species survived all the examined concentrations. On the contrary 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) related to all types of soils. Only the untreated bauxite residue (S) caused meaningful mortality (> 20 %) (Table 7, Fig. 2).

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	[illegible]0.2234	0.00[illegible]
BR	21056.09	1	21056.[illegible]	435.3233	0.000
Soil	1874.25	2	[illegible]37.12	[illegible]3745	0.000
Conc	42168.80	5	[illegible]433.76	17[illegible]35	0.000
BR*Soil	6406.39	2	3203.19	66.224[illegible]	0.0[illegible]
BR*Conc	19241.73	[illegible]	6413[illegible]	132.6042	[illegible]00
Soil*Con	10276.5[illegible]	[illegible]	[illegible].66	21.24[illegible]	0.000
BR*Soil*Conc	516[illegible]	6	[illegible]860.27	1[illegible]6	0.000
Error	[illegible]41	96	[illegible]37		

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

3.3 Motion-frequency tests

The *Enchytraeus albidus* was sensitive to undiluted bauxite residue (100 %). Both types of 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 by both test species. As it was mentioned in Material and methods valid part (2.5), the Chi-Square and *p*-values was less reliable, the graph (Fig. 3) was instructed.

The parameter estimates and the calculated values give more reasonable information about the effects. The expected motion-frequencies were similar in the two 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 for the other (S). If this type (G) was added to the soils, the effect of the treatment depended

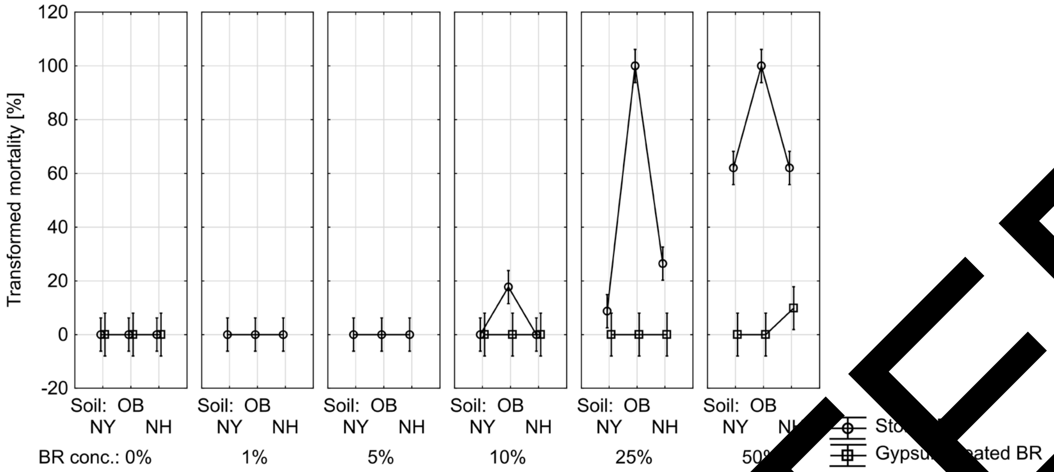

Fig. 2 Transformed mortality of *E. albidus* estimated by the model (with 95 % confidence inte[illegible]s) fo[illegible]ferent types of soi[illegible]e function of added bauxite residue concentration (BR: bauxite residue, NH: Nagyhörcsök, NY: Nyírlu[illegible] OB: Őrbottyán)

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	0.0[illegible]
Soil	2	37411.88	253.8	0.00[illegible]
Conc	3	37360.50	[illegible]	0.000
BR*Soil	2	37507.76	62.1	0.000
BR*Conc	3	37490.9[illegible]	95.7	0.000
Soil*Conc	6	37503.9[illegible]	69[illegible]	[illegible]
BR*Soil*Conc	6	[illegible]2.38	[illegible].8	0.000
		Dendr[illegible]ena veneta		
BR	1	[illegible]66.68	1[illegible]	0.000
Soil	2	1[illegible]1	37.1	0.000
Conc	4	1920.[illegible]	111.7	0.000
BR*Soil	2	1961.98	28.2	0.000
BR*Conc	4	1966.91	18.3	0.001
Soil*Con[illegible]	[illegible]	1947.59	56.9	0.000
Soil*BR*Co[illegible]	8	[illegible]5.33	61.4	0.000

on the t[illegible]e of the s[illegible]. there was an interaction between these [illegible] factors. In silty soil (NH) the motion frequency in[illegible]ased sig[illegible]ntly only at the 50 % dose of BR com[illegible]red to the control soil (C). In sandy soils (NY, OB) the tre[illegible] of the increase in motion frequency was very similar to eac[illegible] other but it differs from the case of silty soil (NH). This tendency was not observable by untreated bauxite residue (S). There is an interaction between the types of bauxite residue and concentrations factors. In addition, it could be observed that the interaction between the soil type and conc. depends on the type of BR (three-way interaction). Con[illegible]ing to the untreated baux[illegible] residue (S), if the acidic san[illegible] soil (NY) was [illegible]sed (instead of NH) the motion-frequen[illegible]y was 1.76 time[illegible] higher (8.55) than in the reference soil. [illegible]e calcareous [illegible]andy soil (OB) generated similar motion-[illegible]uency [illegible]e other sandy soil, the expected value [illegible] the motion- frequency was 8.40. The highest value of m[illegible]quencies was predicted by the 50 % concentration [illegible] 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 behavior in connection with the motion-frequency than in case of *E. albidus*. With stored sample (S) in the silty (NH) and calcareous sandy (OB) soils it resulted approx. 1.7 times higher expected value in the motion-frequency than with acidic sandy (NY) soil. Although the motion-frequency increased by concentration, the effect was influenced by the type of soil and bauxite residue. The test-organism reacted similar motion-frequency

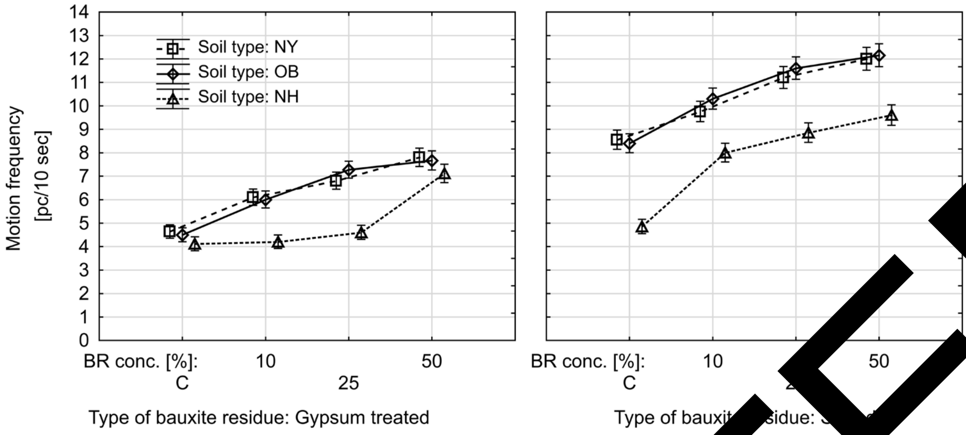

Fig. 3 The LS means plots (with 95 % confidence intervals) of the fitted generalized linear model *E. albidus* (BR: bauxite residue, NH: Nagyhörcsök, NY: Nyírlugos, OB: Őrbottyán)

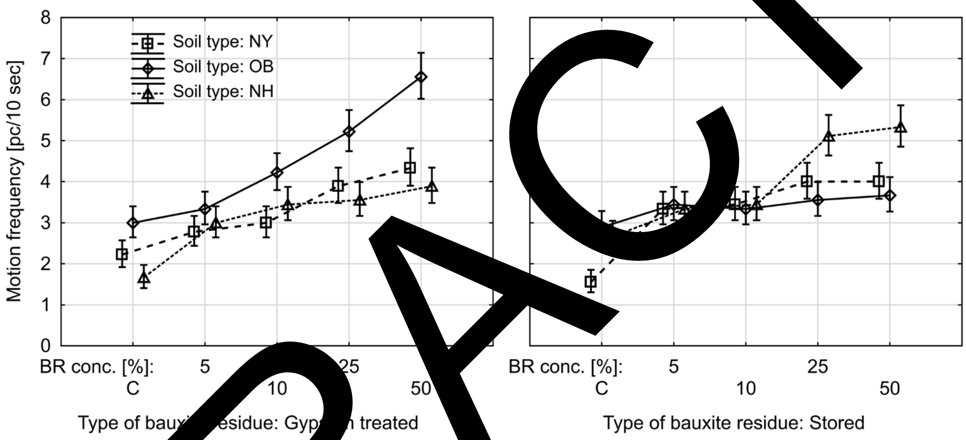

Fig. 4 The LS means plots (with 95 % confidence intervals) of the fitted generalized linear model *D. veneta* (BR: bauxite residue, NH: Nagyhörcsök, NY: Nyírlugos, OB: Őrbottyán)

changes in 5 % bauxite residue concentration as in gypsum treated bauxite residue (G). In case of untreated bauxite residue (S) these concentrations were the 5 % and 10 % added concentrations (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 by other two soils (NH, OB). Related to originally neutral or slightly alkaline soil (NH, OB) the further increasing had no ecotoxicology advantages. Moreover, the bauxite residue addition can rise the water holding capacity of soils [7, 46]. Facing these studies only the higher concentrations (> 10 %) affected in a significant way. On the other hand, the bauxite residue usually contains some potentially toxic concentrations of elements and metals [47–49].

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.

Table 9 Average motion-frequency changing (Stimulating%) with factorizing to gypsum treated experimental series controls (G: gypsum treated-, S: stored bauxite residue, BRX: concentration of bauxite residue, 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

Although the earthworm acute mortality test is recommended characterizing the ecotoxicological [illegible] 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. [25] results. Moreover, it confirms Courtney et al. [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 accordance with Kerekes and Feigl [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*. We confirmed the literature data, 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–[illegible] % soil moisture, the animals can tolerate 4.8–[illegible] 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 Graefe 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].

[illegible] 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 Ujaczki et al. [7, 50], Finngean et al. [28] and Kerekes and Feigl [29] results.

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 in neutral class. The addition of lower concentrations (< 10 %) were safe according to XRF analysing related to all the types of soil.

The untreated bauxite residue (S) caused significant mortality on 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 (25; 50 %). In this research, the bauxite residue was a possible soil-ameliorant material by acidic soil with inefficient water holding capacity. The treated bauxite residue had fewer toxic effects and was safe in bigger concentrations. Therefore, the treated bauxite residue seems to be a better soil ameliorant 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 the utilization as soil ameliorant material, because it may be a well-appropriate reusable option. Furthermore, we should collect more information about the short- and long-term sensitivities of other taxonomic group 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: Sw[illegible]es, 1999 [40]); <DL[illegible]ow Detection Limit, T. H.: Threshold value, S: stored-, G: gypsum treated bauxite residue, NH: Nagyhörcsök, N[illegible] Nyírlugos, O[illegible] Őrbottyán

	As [mg/kg]		Cr [mg/kg]		V [[illegible]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	[illegible]	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	[illegible]	81.8±31.4
10 %	**23.1±1.6**	**23.6±0.9**	**79.9±9.4**	**101.2±4.9**	[illegible]	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**
			N[illegible]			
C	<DL	<DL	19.5±12.2	[illegible]	<DL	<DL
5 %	9.45±1.6	7.2±1.99	[illegible]	[illegible]	66.0±9.6	36.4±0.0
10 %	**15.7±3.0**	14.3±7.0	[illegible]	58.9±27.0	116.9±8.8	73.7±47.3
25 %	**33.0±3.2**	**34.6±0.7**	[illegible]	[illegible]	213.2±21.5	195.8±39.6
50 %	**61.0±3.4**	**57.8±22.7**	[illegible]	**261.4±68.4**	**353.4±3.0**	245.1±18.4
			OB			
C	4.8±0.0	[illegible]	<DL	<DL	33.2±0.0	33.2±0.0
5 %	9.1±1.6	[illegible]	[illegible]	33.2±17.9	74.7±7.2	56.3±27.2
10 %	**19.3±3.9**	[illegible]	**79.3±25.6**	70.2±2.2	141.8±20.4	86.3±15.0
25 %	**30.1±0.9**	[illegible]	**101.1±9.4**	**179.8±44.6**	199.0±11.1	235.4±58.9
50 %	[illegible]	**101.1±2.2**	**199.3±24.4**	**321.6±70.7**	**343.46±53.5**	**365.6±67.8**

Appendix B

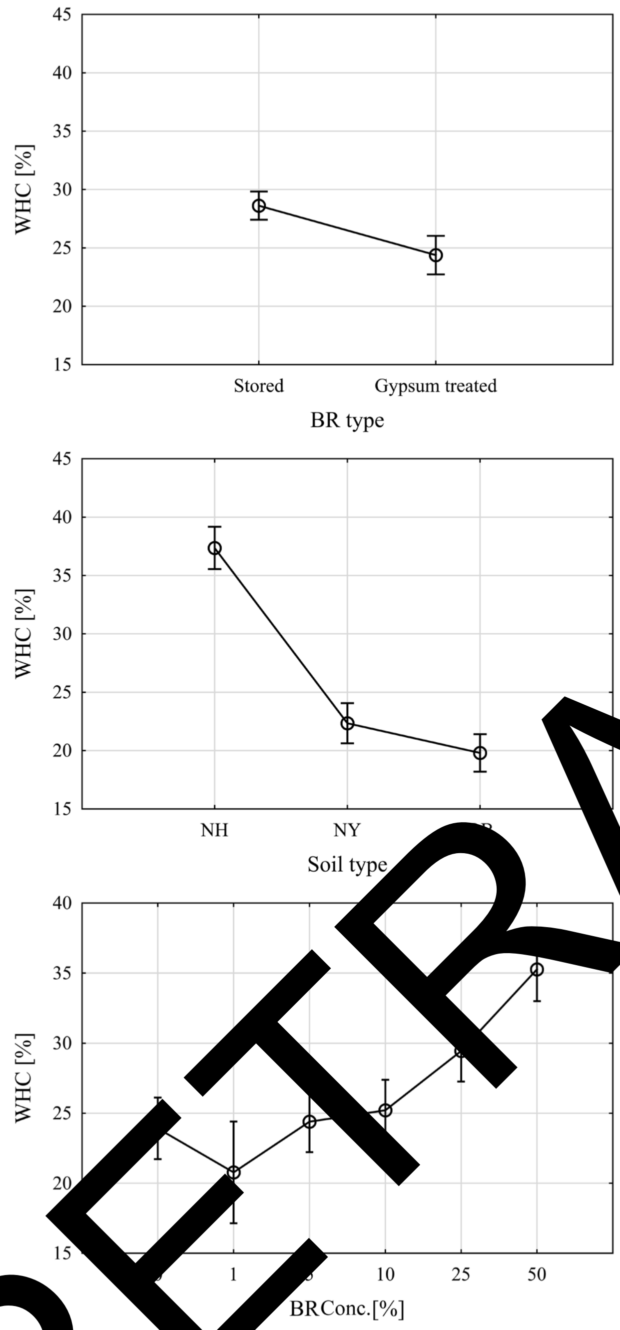

Fig. 5 Weighted means plots of WHC (with 95 % confidence intervals) (BR: Bauxite residue, NH: Nagyhörcsök, NY: Nyírlugos, OB: Őrbottyán)

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±[illegible]	8.5±0.12
OB (C)	3.0±0.06	2.9±0.07	4.5±[illegible]	8.4±0.18
NH (C)	1.7±0.09	2.7±0.07	4.1±0.20	4.9±0.25