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 type had an acute mortality effect (> 25 %). Both species refused the higher concentration soils (≥ 10 %) of both types of bauxite residue. Slight 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, motility frequency, mortality

1 Introduction

The bauxite residue is an industrial waste, which is produced 0.7–2 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] and immobilizes 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 decomposer 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 tested 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.
(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 albidus). The motion-frequency as pre-screening endpoint provides information about energy consumption of organisms. The energy consumption has indirect effect on the survivability 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°18'40.5", 17°32’52.09", E) [30]. The first one was untreated and stored in a deposit (S; pH = 10.4±0.1) 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 slightly sandy soil (NY: pH = 4.9) was originated from Nyírlugos, the calcareous sandy soil (OB: pH = 7.7) was collected from Órbottyán. The silty soil (NH: pH = 7.6) was originated from Nagyhörcsök.

The 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 CaCO₃, c pH [34] (NH: Nagyhörcsök, NY: Nyírlugos, OB: Órbottyán).

| Properties           | NH   | NY   | OB   |
|----------------------|------|------|------|
| Sand:Sil:Clay (%)²   | 17:60:23 | 85:10:5 | 32:5:7 |
| WHC (%)³             | 36   | 30   | 32   |
| Humus (%)³           | 3.1  | 0.5  | 1.0  |
| CaCO₃ (%)³           | 1.8  | 0.0  | 3.3  |
| pH (H,O)³            | 7.6  | 9.9  | 9.9  |

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 | Mixed bauxite residue | Gypsum treated bauxite residue |
|--------------------|-----------------------|--------------------------------|
| pH (pH)            | C; 1; 5; 10; 25; 50; 100 | C; 1; 5; 10; 25; 50; 100 |
| WHC (%)            | C; 1; 5; 10; 25; 50; 100 | C; 1; 5; 10; 25; 50; 100 |
| pH (XRF)           | C; 5; 10; 25; 50; 100 | C; 5; 10; 25; 50; 100 |
| Mortality frequency| C; [5]; 10; 25; 50; 100 | C; [5]; 10; 25; 50; 100 |
| Mortality (%)      | C; 1; 5; 10; 25; 50; 100 | C; 1; 5; 10; 25; 50; 100 |

The untreated natural soils were used as control.

As 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).

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 = 80 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. We called an animal "refused" if 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 Lourenço et al.'s [36] method ) we used a double of their volume which was 20 g/animal dry weight of soil [10 g/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.

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 was 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 per animal was more than 10 seconds, we excluded the animals from the test series.

The motions of the animals were counted for 10 seconds which ended at an acceptable normalization time. Before examining a new organism, we waited that the previous one burrowed the lower layer to avoid influencing each other. We examined 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 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 | 111328.5±3113.1 |
| Cr      | 436.4±40.3 | 349.8±15.0 |
| Cu      | 106.3±15.4 | 112±3.1 |
| Hg      | 943.5±279.4 | 1063.4±216.3 |
| Mo      | 7.5±.06 | 171±2.0 |
| Ni      | 402.8±15.8 | 33.5±33.1 |
| U       | 25.2±2.1 | 27.1±4.8 |
| Zn      | 83.4±2.2 | 100.7±7.4 |
| V       | 530.3±103.0 | 709.2±94.0 |

The NY soil had the lowest pH level. The other two soils (OB, S) 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 a reduction in pH level than the gypsum treated (G) one. Only 1% untreated bauxite residue (S) addition raised 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
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: Örbootyán, G: gypsum treated-, S: stored bauxite residue)

|            | NH  | NY  | OB  |
|------------|-----|-----|-----|
| As         | C   | C   | 10% |
| Cr         | 5%  | 10% | 10% |
| V          | 25% | 25% | 25% |
| NOEC       | C   | 10% | 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| 435.233| 0.000|
| BR         | 21056.09| 1              | 21056.09| 1035.323| 0.000|
| Soil       | 1874.25| 2              | 937.12| 17.3745| 0.000|
| Conc       | 42168.80| 5              | 8433.76| 19.735 | 0.000|
| BR*Soil    | 6406.39| 2              | 3203.19| 66.226 | 0.000|
| BR*Conc    | 19241.73| 3              | 6413.9| 132.6042| 0.000|
| Soil*Conc  | 10276.56| 5              | 2055.32| 21.245| 0.000|
| BR*Soil*Conc| 5195.60| 6              | 866.92| 17.386| 0.000|
| Error      | 4941.96| 96              | 51.7|      |      |

There was no death expected by the model in control soils (≤5 %). In case of other two types of soils (NH, NY), 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 prepared 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

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 the case of sandy soils (NY, OB) with originally lower WHC.
There was 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).

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 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

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

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**Table 8** Type III likelihood-ratio test for the main effects and interactions on motion-frequency of E. albidus and D. veneta

|                  | Degr. of Freedom | Log-Likelihood | Chi-Square | P      |
|------------------|------------------|----------------|------------|--------|
| E. 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                | 37490.92       | 95.7       | 0.000  |
| Soil*Conc        | 6                | 37503.95       | 69.6       | 0.000  |
| BR*Soil*Conc    | 6                | 37492.38       | 92.8       | 0.000  |
| D. veneta        |                  |                |            |        |
| BR               | 1                | 1966.68        | 18.7       | 0.001  |
| 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  |
| Soil*BR*Conc     | 8                | 1945.33        | 61.4       | 0.000  |
changes in 5 % bauxite residue concentration as in gypsum treated bauxite residue. 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 \textit{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 \textit{E. albidus}.

Discussion
According to our results the addition of bauxite residue in a low concentration (S: 5 %; G: 10 %) could improve the environmental circumstances of \textit{Dendrobaena veneta} and \textit{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.
Although the earthworm acute mortality test is recommended characterizing the ecotoxicological risks of different types of waste [51], we found that the bauxite residue addition had no acute mortality effect on earthworms. This result fits to Maddock and Forstner [28] results. Moreover, it confirms Courtney et al. [26] results, who did not find acute toxic effects in case of Holobophora communica and A. longa (especially in case of 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 influences 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 acidic or sandy soils. Since the soil pH can be a limiting factor on earthworm distribution [55], the increasing effect can be positive in acidic soil. Although the E. albidus pH optimum range is 6.8–7.0 with 35–40 % soil moisture, the animals can tolerate 4.8–9 pH [56]. Despite this result, we found that the animals could survive higher pH level (max. ~9 pH) in a short time. It is in higher accordance with Graefe and Schmelz [57] results, that this species tolerated slightly acidic 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 wider pH range (e.g. Eisenia fetida 5–9 soil pH [59]).

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

### Table 9

|          | 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**

|          | NH     | NY     | OB     |
|----------|--------|--------|--------|
| G-BR5    | 147.4±11.1 | 125.9±33.6 | 149.1±22.7 |
| G-BR10   | 7.2±4.7  | 7.9±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 |

5 Conclusion

The bauxite residue addition could improve the properties of acidic or degraded 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 re usable 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: Swarties, 1999 [40]); <DL: below Detection Limit, T. H.: Threshold value, S: stored-, G: gypsum treated bauxite residue, NH: Nagyhörcsök, NY: Nyírlugos, OB: Őrbottyán.

| T.H  | As [mg/kg] | Cr [mg/kg] | V [mg/kg] |
|------|------------|------------|-----------|
|      | S          | G          | S          | G          | S          | G          |
|      | 15         | 75         | 150        |
| C    | 11.0±0.2   | 11.0±0.2   | 35.2±7.9   | 35.2±7.9   | 11.0±2.2   | 41.0±2.2   |
| 5 %  | 18.5±1.4   | 17.4±4.1   | 81.0±16.4  | 53.8±14.4  | 9.1±3.48   | 81.8±31.4  |
| 10 % | 23.1±1.6   | 23.6±0.9   | 79.9±9.4   | 101.2±4.9  | 115.0±10.9 | 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 |
|      | NH         |            |            |            |            |            |
| C    | <DL        | <DL        | 19.5±12.2  | 19.5±12.2  | <DL        | <DL        |
| 5 %  | 9.45±1.6   | 7.2±1.99   | 34.6±0.7   | 34.6±0.7   | 56.0±0.7   | 56.0±0.7   |
| 10 % | 15.7±3.0   | 14.3±7.0   | 101.1±18.0 | 101.1±18.0 | 116.9±8.8  | 73.7±47.3  |
| 25 % | 33.0±3.2   | 34.6±0.7   | 101.1±16.4 | 101.1±16.4 | 213.2±21.5 | 195.8±39.6 |
| 50 % | 61.0±3.4   | 57.8±22.2  | 241.4±21.5 | 261.4±68.4 | 353.4±3.0  | 245.1±18.4 |
|      | NH         |            |            |            |            |            |
| C    | 4.8±0.0    | 5.0±0.0    | <DL        | <DL        | 33.2±0.0   | 33.2±0.0   |
| 5 %  | 9.1±1.6    | 10.4±4.2   | 33.2±17.9  | 74.7±7.2   | 56.3±27.2  | 56.3±27.2  |
| 10 % | 19.3±3.9   | 17.2±2.2   | 79.3±25.6  | 70.2±2.2   | 141.8±20.4 | 86.3±15.0  |
| 25 % | 30.1±0.9   | 40.1±3.3   | 101.1±9.4  | 179.8±44.6 | 199.0±11.1 | 235.4±58.9 |
| 50 % | 59.6±0.9   | 101.1±2.2  | 199.3±24.4 | 321.6±70.7 | 343.46±53.5 | 365.6±67.8 |
Table 11 Average, original motion-frequency of different test species in different (NH: Nagyhőrcsök, NY: Nyírlagos, OB: Órbottyán) 

|                      | *Dendrobaena veneta* | *Enchytraeus albicus* |
|----------------------|----------------------|-----------------------|
|                      | 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              | 4.3±0.18       | 8.4±0.18             |
| NH (C)               | 1.7±0.09             | 2.7±0.07              | 4.1±0.20       | 4.9±0.25             |

Fig. 5 Weighted means plots of WHC (with 95% confidence intervals) (BR: Bauxite residue, NH: Nagyhőrcsök, NY: Nyírlagos, OB: Órbottyán)