Hungarian University of Agriculture and Life Sciences

Optimalization of ringworm (Annelida) acute ecotoxicology testmethod to qualify soil as habit, the examining of bauxite residue as soil ameliorant material – case study

Thesis of doctoral dissertation

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1. Introduction and objectives

The bauxite residue is an industrial waste of aluminium production, which is produced approximately 120 million tons per year (http1). Depending on the raw material from which the aluminium is made and producing-process, the properties of the resulting bauxite residue may be different, but in general, all bauxite residue is characterized by a strongly alkaline pH, a high-water-holding capacity and a high content of inorganic elements.

Despite the material is able to be used in industry or as soil- amendment material (Power et al. 2011; Ferguson, 2014; Evans, 2016), these types of reusing are not common. Most of the produced amount is stored as a waste, but there is a high risk of accidents when being stored, both the environmental- and for the human health (Yuzhakova et al. 2013).

The soil acidification is an issue, which effect on 30% of Earth (Kunhikrishnan et al. 2016). Reusing the bauxite residue as a soil-ameliorant material is not only cost-efficient way to improve the soil-health, but this way is able to also reduce the risk of storing (Kunhikrishnan et al. 2016; Ujaczki et al. 2016). One of my objectives is examining that how the soil improvement with bauxite residue is able to influence the soil with bad water-holding-capacity or/and acidic pH on the habit of ringworm population.

Since the treatment of the bauxite residue could be different (e.g. neutralization), therefore my experiments were conducted with two types of it: an untreated (derived from storage area, 2016) and a treated one (2% gypsum neutralized, 2016).

According to the literature, the potential toxicity of bauxite residue is different related to different soils (Sanderson et al. 2014), thus I examined these residues with mixed three different, Hungarian, natural soils (acidic sandy-, neutral sandy, neutral silty soils).

Taking into consideration the fact, the free-living species of annelids have variant body-size, the ecotoxicological tests were made with both large (*Dendrobaena veneta)* and small (*Enchytraeus albidus*) species.
Since free-living ringworms are able to actively migrate from an area, I considered it necessary to work in addition to the traditionally used standardized test methods, a new, alternative endpoint to obtain more information about the sub-lethal effects of xenobiotics.

The introduction of this alternative endpoint was with modifying and completing the existing standard test methods after the examining its applicability. Because of the above-mentioned reasons, I have two main goals with the objects: test-method improving and completing, and the examining of bauxite residue as soil-amendment material with annelids' ecotoxicological tests in case of natural soils.

I.1. New endpoint (motion-frequency) development which offers extra information about the escape behaviour, so active mitigation of animals during the traditional lethal or reproduction inhibition: Examining whether the endpoint measurement can be repeated with sufficient precision. Furthermore, I would like to examine that whether the method devised for measuring the endpoint can be applied with sufficient reliability.

Objectives related to the assessment of the environmental toxicological effects of bauxite residue: II.1. Investigation of the change of their physical and chemical properties (pH, water-holding-capacity, metal-content) by adding bauxite residue to the soil. II.2. Assessment of the acute lethal and sublethal effects of bauxite residue with using common potworm (*E. albidus*) and earthworm (*D. veneta*) test-species.

2. Material and methods

2.1. Examined bauxite residue and soils

During the examining-period, I focussed on two bauxite residue from a non-operating Hungarian factory (N: 47°5’19”; E: 17°32’52”): untreated - from storage area (T) and treated sample, which was neutralized with 2% gypsum after dewatering. OECD artificial and three natural soils from Hungarian Academy of Sciences-Centre for Agricultural Research sample area were used: Nagyhörcsök (NH, pH=7.6, silty), Nyírlugos (NY, pH=4.9, sandy), Őrbottyán (OB, pH =7.7, sandy).

2.2. Test organism

The annelid with higher body-size, *Dendrobaena veneta* (Rosa, 1886) test-organism were derived from special (living bait) shop. The species identification was made by expert of Department of Biology, Strossmayer University (Davorka Hackenberger) in 2015 before the starting of experiments. Before testing, animals had been conditioning for 14 days in their original medium, dark, then they had been starving for 48 hours in filter-paper covered boxes.

The ovuled female common potworms (*Enchytraeus albidus* Henle, 1837) of maintainer stock cultures were derived from stock cultures (2016) of Hungarian University of Agriculture and Life Sciences (formerly Szent István University). Mixed cultures were made for the testing from the synchronic cultures. Only by OECD accepted adult individuals with minimum body-length 1 cm were used.

2.3. Applied chemicals, labour materials and programs

Filter paper was used in accordance with the current standard. Chopper-sulphate dilution was used in case of experimental-series which examined the reproducibility and acceptability of motion-frequency test. Distilled water was used as solvent material and watering in ecotoxicological tests. The diagrams were made by Microsoft Excel, a TIBCO Statistica 13.4 and a Rx64 3.4.1 programs in the dissertation.

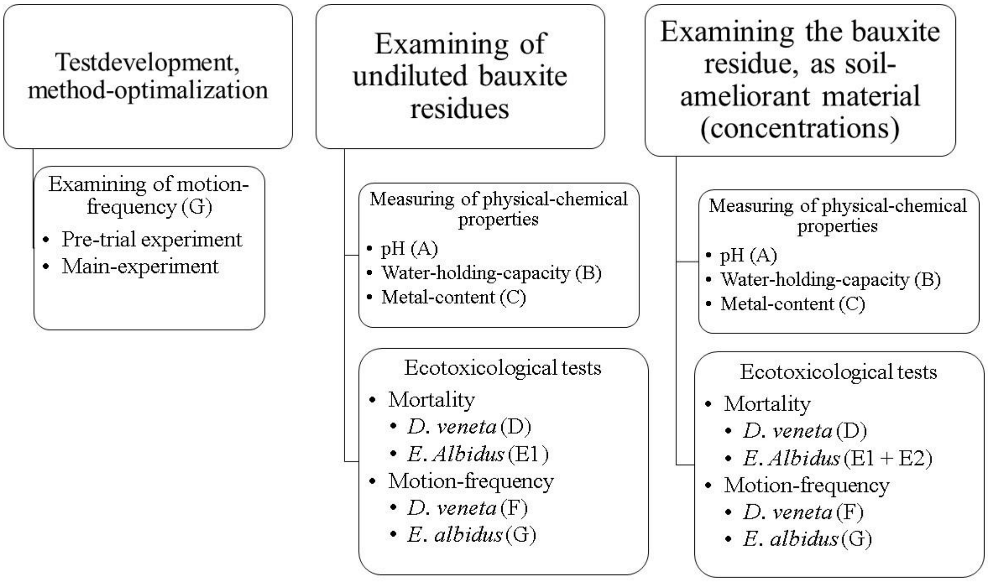
2.4. Sample preparing

The solid bauxite residue was dried to total anhydrous state in glass-plate at room temperature. Dried samples were ground in mortar, then sieved with a 2 mm hole diameter sieve. Materials were displaced from samples which were not able to be ground. The process was repeated when the final material was become fully homogenous powder. The powdered form of untreated soils was used as control material. The setting of concentrations was mixed by weight measuring from the powdered samples. Besides the solid samples were also soil-extracts and -

suspensions examined in some experiments. Suspensions and extracts were made from powdered samples. MSZ 21978-9:1998 standard was used in both cases.

2.5. Comprehensive description of the performed experiences

The experiments were made in the labours of MATE and BME during 2017-2021. The experimental set-ups were introduced in 1. Figure.



1. figure- Performed experiments in each series
(x): sign of applied test-method

OECD soil was applied just to develop the motion-frequency test. To evaluate the method of the motion-frequency test, the precision of the test (Gage R&R test) and its applicability were measured. The accuracy involved the precision repeatability over time (accurate at multiple times) and reproducibility (accurate by multi-person). How similar ecotoxicological conclusions were deducted to investigate of acceptability. Model (OECD) soil and $CuSO_4$ reference pollution were applied by measurements. 0, 24, 48, 60, 96, 120, 240, 2400 (preliminary experiment) and 0, 24, 48, 60, 96, 120, 240 (main-experiment) mg/kg concentrations were investigated. The valuing of main-experiment was made from results of 3 observers in 2 times with 3 weeks different.

The untreated silty soil (NH-K) served as a control in the case of undiluted bauxite residue, as this had the most appropriate conditions for annelids according to literature data (pH, water-holding-capacity). Both types of bauxite residue with natural soil mixed forms were investigated the listed tests (1. figure). Only the examining of three forms (powdered-, soil-suspension and -extract) was exception, which was made only with the soil-mixed concentrations of more toxic bauxite residue. The concentrations of bauxite residue were made in one amount at the start of the experimental phase at same time, once. The three replications were derived from the same mixing. Replications were made and examined in rapid order during the experiments.

2.6. Measuring of physical and chemical properties

A: pH measuring was made by MSZ 21470/2:1981 standard.

B: The measurement of water-holding-capacity was carried out by the modified version of retracted MSZ 21470-2:1981 standard. 3×25 g of each powdered samples was placed in a 2 cm diameter plastic tube with a water permeable membrane. Samples had been placed in water bath for 1 hour, then on a sand bed for 3 hours. After 2 g of amount from all the tubes was measured and put to jars, the jars were spent 24 hours in 105°C heat drying chamber. After drying, all the jars were measured again, and the mass differences were used for calculating the water-holding-capacity.

C: The metal-content was measured with NITON XL3t 600 (Thermo Fischer Scientific) portable X-ray fluorescens spectrometry machine. After calibration, the machine was carried out the analysis of 33 different elements with 135 s/sample running time.

2.7.Mortality test methods

D: The test method was based on OECD 207 guide related to higher test-species (*D. veneta*), but the amount of sample was optimized to 40 g/individuals. Glass jars were used which had 10 cm diameter and 500 ml volume. After 120 g soil measuring, the soils were watered to 60% of water-holding-capacity. The animals were placed on these watered soil samples. 3-3 individuals were tested in each replicates. The jars were stored protected from light in 20±2 °C. The jars were inspected in 7 and 14 days. Dead animals were counted. Moreover, the „refusing" as a behavioral abnormality was noted too: if the individual was still moving to a mechanical stimulus (so were alive), but it stayed on the top of the soil.

E1: 20 g soil sample was measured in all vessels in case of the common potworm (*E. albidus*) test species, which was watered to 60% of water-holding capacity. 6 cm diameter glass jars were used to assess the lethal effect of bauxite residue. 5 animals were placed on the watered soil. The tests were stored in dark, 20±2 °C. According to OECD 207 guide, 7 and 14 days inspection times were applied. The number of both the dead and the "refusing" behaviour deviance were noted.

E2: Not only the solid-, but also the suspension and extract forms were examined. All the three forms were examined related to all the three types of soils. The solid samples were measured also in a 9 cm diameter glass Petri-dishes. According to Amorim et al. (2005) work, 25 g sample was taken in all Petri-dishes. In this case, the samples were moistened to 60% of the water-holding-capacity. The examining of suspension and extracts were carried out in the previous described glass Petri-dishes, which were covered with laboratory filter paper. 3 ml sample was flowed in the filter paper. All Petri-dish tests were made protected from direct light at constant temperature (21±1°C) during 96h exposition time. Dead individuals were counted at the end of the test.

2.5. Measuring of motion-frequency endpoint

The relocating behaviour was estimated by the definition of peristaltic locomotion in Kristian's (2017) work. During peristaltic locomotion, 3-8 rings are shrieked at same time which are formed a „temporary leg". This shrinking area is started to relocate for the anterior part of the body. Meanwhile the segments before the shrieked area are stretched, pulled up the front rings and pushed forward the pulled-up part. More contractilicted parts are moved at same time in more cases.

F-G: One motion was the unit where the contraction (leg) was gone along the full body-length, all rings. The unit was consequent according to each observer in the tests which were made by more observers.

The motion-frequency endpoint was the part of the mortality test in case of examining of both, the undiluted bauxite residue and bauxite residue concentrations. The top of the soil was smoothed by both test-species. One animal was placed in the test-vessel by the examining of endpoint, the motion of this was measured 2×10 s long after the habitation time. Habitation time (1-10 s) was the period, when the peristaltic-motion of animals became consistently rhythmic. If this did not happen, the animal was rejected and removed from the test.

2.9. Analysing of results, statistical analysis

The conditions of applications were separately verified in all cases. Significant difference was $p < 0.05$. Because the applied tests were to sensitive due to the number of items, the F-values by the ANOVA models (part of the GLM model too) and the Chi-square probe statistic values were offered estimation to the size of changes.

2.9.1. Examining of motion-frequency endpoint

Gage R&R study was applied to evaluate the precision of method. 2 factors were included by ANOVA test: the concentration and test vessel. The reproducibility was evaluated by basing on the results of the main experiment' first test. The effects of 3 factors were tested with ANOVA: the concentration, the test-vessel and the observer. The repeatability was examined with the comparison of the results of the 2 tests with 3 observers. A new factor was built in this model: the day. To numbering of the precision of the 2 tests was calculated standard-deviation-percentage:

The applicability (accuracy) of the tests was evaluated with more approaches. Stimulation% was calculated based on inhibition-percentage by tests.

$$\text{Stimulation\%} = \frac{\text{Average motion (in the concentration)}}{\text{Average motion (in the control)}} * -100$$

The stimulation% as normal covariant was valued with multi-way ANOVA, in which the day, the observer and the concentration were factors. The ANOVA test (with Tukey-test) was able to do in the part of GLM model because of the structure of data. Ring tests were imitated by the selection of data to take a closer approach to the common practice (the data was separately tested in case of different days and observers. LOEC and significant concentrations were applied to the valuing of this view. In this case, the data was separately examined with ANOVA test. Similarity [%] was calculated to compare the ANOVA groups. This number was showed that, how many cases were statistically same changes detected.

2.9.2. Valuing of physical- and chemical properties

A: Comparing to undiluted bauxite residue samples was 2 sampled t-test used. USDA (US. Department of Agriculture) categories were applied to classify the pH of soils.

B: Comparing to water-holding-capacity of undiluted bauxite residues was 2 sampled t-test used. Multi-way ANOVA test was applied to detect which factors were able to influence the water- holding-capacity by analysing of measured value and the concentrations. NH-K was the reference level.

2.9.3. Valuing of mortality tests

D-E1: 2×2 frequency table was applied to examine the undiluted bauxite residues related on both test-species. In case of the bauxite residue concentrations (as soil-ameliorant material) was tested the mortality (co variant) with multi-way ANOVA after the normalizing of data. Archus-Sinus transformation was applied to normalizing.

E2: The lethal effect of bauxite residue with different forms was valued with multi-way ANOVA related to *E. albidus*. The test was applied by the normalized data with Archus-Sinus transformation. The comparing of average lethal effect was made by Fisher LSD probe.

2.9.4. The valuing of motion-frequency test in case of bauxite residue concentrations

Same test method was applied to valuing the motion-frequency results in case of both test species.

F-G: The motion-frequency was handled as Poisson distribution data by both the undiluted samples and the concentrations. Poisson-regression (GLZ model) was adapted to analyse. The concentration was a continuous co-variant. The average value of untreated silty soil (NH-K) was the reference level related to both the undiluted bauxite residue and the concentrations. Stimulation% was calculated to better compare the effect of both the undiluted bauxite residue and concentrations.

3. Results and discussion

3.1. Motion-frequency endpoint

I found by the examining of motion-frequency endpoint with Gage R&R method that, there were any severe differences between the two measuring times in case of precision. Only the measuring day (so different culture) was able to raise the additional component of variance, the observer and test-vessels had no similar effect.

1. table: Component of variance in case of 10 and 20 s measuring times

According to the results during 10 s measuring		According to the results during 10 s measuring	
Factors	Variance-component	Factors	Variance-component
Test-vessel	0,004	Test-vessel	0,009
Observer	0	Observer	0,001
Day	0,088	Day	0,177
Obs. × Conc.	0,001	Obs. × Conc.	0,002
Day × Conc.	0,005	Day × Conc.	0,010
Obs. × T.-vessel	0	Obs. × T.-vessel	0
Obs. × Day	0,000	Obs. × Day	0,000
Error	0,024	Error	0,028

The standard deviation was 10-16% related to both measuring times. Based on this value, the method was acceptably reproducible, but the effect of the day of measurement was decisive.

The ecotoxicological conclusions were same in greater proportion among the observers in case of 10 s measuring time. Average reproducibility was 85% concerning to the ecotoxicological viewpoint (Similarity [%]: 10 s measuring time: LOEC 83; significant differences from control in concentrations: 92; equal effects of concentrations: 79).

Although the results of test were also properly assessed with 10 s measuring time concerning to ecotoxicological viewpoint, there were differences related to the day. This difference should be taken into consideration in the future applications and the results of motion-frequency test will be adapted as estimation (pre-screening results) or evaluated together with results of different methods.

3.2. Undiluted bauxite residue samples

The treatment of bauxite residue was able to significantly decrease the pH (T: 10.4±0,1; G: 9.4±0,0) and water-holding-capacity ([%]: T: 51.8±3.4; G: 33.4±1.0). According to 50/2001 (IV. 3.) government decree about sewage sludge, the arsenic, the cobalt, the chromium, the mercury and the nickel were presented in higher amount than threshold. Furthermore, both types of bauxite residue had high vanadium-content.

Significant lethal effects were detected in case of both test-species. The individual tolerances of *Denrobaena veneta* (average surviving [%]: G: 22.2±38.5; T: 55.6±50.9) were different. The common potworm (*Enchytraeus albidus*) was very sensitive to untreated bauxite residue (average surviving [%]: G: 93.4±11.5; T: 0.0±0.0).

The undiluted bauxite residues were able to influence the behaviour of animals in sublethal way too. The untreated bauxite residue was inclined with a greater response in case of both test-species, than the treated one (Stimulation [%]: *D. veneta*: G: 58.3±7.0; T: 70.8 ± 7.9; *E. albidus*: G: 137.4±0.0; T: 141.6±19.7). The undiluted samples were refused by both test species. According to the results, the fact of literature (Evans, 2016) will be clarified that the treatment of bauxite residue is able to change the physical-chemical, and also ecotoxicological properties of its.

3.3. Bauxite residue as soil-ameliorant material (concentrations).

2. table- Ecotoxicologically acceptable concentrations

		NH		NY		OB	
	Acceptable	T	G	T	G	T	G
pH	max 7,8	K	K	5	10	K	1
Element-content	<threshold	K	K	5	10	5	5
D. veneta mortality	max 20%	>50	>50	>50	>50	>50	>50
E. albidus mortality	max 20%	10	50	25	50	10	50
D. veneta motion-frequency	max 25% increasing	5	5	<5	5	10	5
E. albidus motion-frequency	max 25% increasing	<5	<5	<5	50	<5	50
Maximum acceptable examined concentrations		**K**	**K**	**5/<5**	**5**	**K**	**1**

The bauxite residue addition raised the pH in significant and concentration proportionately way. The different types of soil and bauxite residue were able to influence this effect. The water-holding-capacity was significantly changed by the bauxite residue addition. The size of the effect was influenced by properties of bauxite residue and soils in also this case.

The lowest concentration increased higher the content of elements than threshold of the 6/1999 Government Decree (As, Cr) and Swarties (1999) (Va). Depended on the types of bauxite residue, maximum 5% concentration (in case of OB) or 10% concentration (in case of NY) was adaptable (2. table)

The test-species with bigger body-size (*D. veneta*) survived to all the examined concentrations. Only one-one individuals were died from the concentrations of NY-10% untreated and NY-50% gypsum treated concentrations. Higher concentrations were not preferred by the individuals. These higher concentrations were totally (100%) refused by them (G≥25%; T≥10%) in case of all the three examined soil types.

The common potworm (*E. albidus*) was more sensitive to the bauxite residue than examined earthworm species. Only 10% concentration was able to cause significant mortality in case of the solid form. The suspension and extracts were not able to make an influence in this way.

Mixing the bauxite residue with soils affected in a sublethal way too (3. table).

3. table: Average stimulation [%] (± stand. dev.) compare to the value of each controls

	NH		**NY**		**OB**	
	G	T	G	T	G	T
Dendrobaena veneta						
5%	12,5±2,4	25,0±2,5	19,1±2,6	171,4±34,3	11,1±1,1	20,0±4,0
10%	35,4±0,9	29,2±1,6	38,1±2,3	182,1±20,3	40,7±1,9	16,0±2,7
25%	33,3±1,8	91,7±3,5	66,7±8,7	235,7±19,6	70,4±9,6	24,0±2,6
50%	45,8±2,3	95,8±7,1	85,7±6,6	246,4±11,5	118,5±7,0	28,0±2,5
Enchytraeus albidus						
5%	147,4±11,1	36,3±2,1	125,9±33,6	4,7±0,2	149,1±22,7	8,5±20
10%	2,7±4,7	55,1±2,3	78,9±5,6	7,6±0,3	89,5±10,5	12,2±0,8
25%	12,2±2,3	69,9±2,7	100,0±8,4	16,7±0,8	130,7±15,4	20,5±0,9
50%	73,0±9,4	83,0±4,5	128,5±5,1	21,8±1,3	103,5±42,5	24,1±1,2

The information of 3. table was confirmed this trend, the intensity of motion was become higher with raising the concentration in case of both test-species, the rates of this stimulation depended on the soil- and bauxite residue type.

As current results show, the concentrations of treated bauxite residue had less toxicity when it had mixed with soils. The smaller concentrations of both bauxite residue had severe soil-ameliorant effect when it had mixed with acidic (NY) soil.

The examined concentrations (0-50%) had no significant lethal effect on test-species with bigger body-size (*D. veneta*), which were in accordance with the findings of Maddock et al (2005)', in contrast with other results (Sanderson et al. 2014; Hackenberger et al. 2019, Courtney et al. 2020; Di Carlo et al. 2020). To my mind, the main reason of this trend was that, the different bauxite residue had different properties.

The complex mixing-toxicity effect on individuals of common potworm (*E. albidus*) species was not classified by basing on the data of literature, because there have been any published information about it, therefore, it has not been excluded this as the reason of toxicity.

The pH of soils can influence the surviving and reproduction of annelids (Peijenburg et al. 2012). The pH increasing effect of bauxite residue may have been positive by acidic soils, because the pH optimum of common potworm (*E. albidus*) is 6.8-7.0 (tolerance range: 4.8-7.4) (Chapman et al. 2013).

The greater test-species (*D. veneta*) prefers the slightly neutral pH level and high water-content (67.4-84.3%) (Smith and Stringfellow, 2010). Although I have not found information about pH tolerance of this species, the concentrations were not able to cause significant mortality, because the earthworm-species have typically wide tolerance (pH:5-9) (Edwards and Arancon, 2005).

Briefing my results, typically 5% bauxite residue amount had any severe toxic-risk, but the peristaltic motion-frequency, as the most sensitive (behaviour) endpoint was detectably influenced by also this concentration. These results were in accordance with the results of Finngean et al. (2018).

Evaluating together both the results of mortality and motion-frequency tests, the trend, that the soil-properties (e.g.: characteristic particle size; real water-content) were able to influence the behaviour of annelids, was verified (Ruiz and Or, 2018).

The previously presented results were in accordance with the information of literature (Ujaczki et al. 2016), according to which maximum 5% applied bauxite residue concentration might have been a soil-ameliorant material without severe ecotoxicological risk.

I can verified this results with acute ecotoxicological test (*Sinapis alba, Triticum aestivum, Tetrahymena pyriformis, Dendrobaena veneta, Folsomia candida*) in microcosm experience (untreated bauxite residue mixing with acidic-(NY) and carbonated (OB) sandy soils) which is not a part of my dissertation (Feigl et al. 2017; Kerekes et al. 2017a; Kerekes et al. 2017b).

According to previous written results, maximum 5% bauxite residue is able to improve the properties of acidic sandy soils which are important to annelids, so this addition is able to ensure better habit for animals.

4. Suggestions and conclusions

The presented method optimization experiments were successful, but further repeats of test with generally known groups of pollutions (organic compounds, water unsolvable materials, nanomaterials, microplastics) is also pivotal to pronounce about that, the motion-frequency test is widely and reliably acceptable.

Moreover, real-ring test will be essential according to my view. If it should verify that, the test is repeatable with all common annelids, this test should been repeated with other well-known test species (*Enchytraeus crypticus, Lumbricus terrestris, Eisenia fetida*). Besides ring-test, the effect of the day (state of the testing population) should been better explored. To this goal, further experiments have to been accepted to detect the additional variance effect of testing population (day).

In my opinion, although the examining of motion-frequency with special equipment will been become the test-method only harder adaptable for the labours with minor financial resources, the investigation of method's targeted technology (recording equipment and analysing software) is useful. In this way, the additional variance of the observer and the limited repeatability can be avoided.

Many questions must be answered to use the bauxite residue as soil-ameliorant material: It is important to clarify, how long the pH increasing effect is prevailed. I suggest long term (years long) field experiments to investigate this. I think, it is necessary to detect the potential risk of bauxite residue's toxicity (e.g. metal-content) after reacidification as a part of the long-term field experiments.

To understand the risk, the detection of the effects of metals' different forms and complexes it is also necessary related to examine test species. It should clarify how big amount of metals are available. As I see, the question should examine, which is the reason of the high standard deviation in the mortality test with test-species with greater body-size (*D. veneta*) related to undiluted bauxite residue samples.

Only the solid powdered form of bauxite residue had significant lethal effect. Probably, the reason was the NaOH molecule, which, in high amount, could be responsible among others for the alkalinity of bauxite residue. Natrium-hydroxide is absorbent which is able to keep high amount of water. According to my opinion, the higher toxicity of solid form was caused not only by the increasing pH level, but the extracted water from the cuticules of animals by natrium-hydroxide. To verify this, further histology tests are needed.

Despite the fact that the extracts and suspensions had no significant toxic effect on potworms (*E. albidus*) in standard dilutions, it is needed the examining of thicker concentrations and even after exploration with other solvents (e.g., with mild organic acids) to investigate the issue to better model the potential toxic effects of groundwater in intervention areas for later use.

It is important to investigate, the bauxite residue and metal tolerances of the two examined test species *(E. albidus* and *D. veneta*) are appropriate model for the investigation of sensitivity of the families (Enchytraeidae and Lumbricidae).

According to my data and the information of literature, the sensitivity of groups and the taxons in the groups are really different. Before using the bauxite residue as soil-ameliorant material, it is important to assess the tolerance of further, sensitive soil-living test-species (e.x. K-strategic nematodes, collembolas, different snakes and spiders)

Before the application in practice, modelling the potential chronic and long-term effects on population of annelids is essential. Besides the guided OECD 220 and 222 reproduction inhibition tests, the avoidance behaviour and the transgenerational effects (changing/ deformity during growing, enzymatic experiences) are recommended as my view. As my opinion, the soil-avoidance or selection test is able to be suitable model to detect exactly how much with soil-ameliorant bauxite residue has soil improvement properties compared to the original soils.

The sensitivity of species can be different in the soil-ecosystems. Because of this, I need the previously environmental risk estimation completed or optimized with the properties of the area is indispensable before each application.

If we would use safety the bauxite residue in the practice, I consider it of paramount importance to explore whether soil-improvement with bauxite residue influences the effects of different agrochemicals: when these keep in interaction, there are potential toxicity from phenomenon of synergism or potentiation.

5. New scientific results

I.1. New endpoint (motion-frequency) developing was the goal which offer extra information about the escape behaviour, so active mitigation of animals during the traditional lethal or reproduction inhibition: Examining whether the endpoint measurement can be repeated with sufficient precision. Furthermore, to examine whether the method devised for measuring the endpoint can be applied with sufficient reliability.

According to my currently available results, the motion-frequency test is carried out as the part of mortality tests (OECD 207 and 220) with sufficient precision and accuracy (repeatable and reproducible). Using with the 10 s measuring time, it is suitable to give extra, pre-screening information beside the results of the common standard test. The observer influenced, but the ecotoxicological conclusions were become more distorted in just acceptable level (<average 20%) compared to daily control.

In contrast with the traditionally applied method, pre-screening results almost immediately are given by the observation of motion-frequency. It is important to note, compared to each other might the individual sensitivity of the different synchronic-culture be different in different times and the observer can be influenced the measured results, so the appropriate selection of control is essential to compare the test-results. Based on the current results, the test-methods were able to successfully apply with also both potworm (*E. albidus*) and earthworm (*D. veneta*) test-species.

Kerekes, I. K., Pusztai, É., Feigl, V., Kemény, S. (2022): Acute ecotoxicological effects of bauxite residue addition on mortality and motion-frequency of *Denrobaena veneta* and *Enchytraeus albidus* (Annelida) in three types of soils. Periodica Polytechnica Chemical Engineering, https://doi.org/10.3311/PPch.19868

Pusztai, É., Kerekes, I., Hegyi, Z., Kemény S. (2021): Gage R&R study a környezettoxikológiai vizsgálatokban (presentation). KeMoMo–QSAR 2021 szimpózium, 2021 szeptember 30-október 1. Szeged.

II.1. I would have liked to investigate that, how change the physical and chemical properties (pH, water-holding-capacity, metal-content) by adding bauxite residue to the soil.

Face with the information of literature, that the bauxite residue is an appropriate material to improve the quality of sandy soils, according to the published results of my PhD-dissertation, other, peer-reviewed, scientific works and scientific works which made by my participation (Feigl et al. 2017, Kerekes et al. 2017a), the safety application to soil-ameliorant was based on the original properties of examined soil (pH, element-content) and bauxite residue (pH, element-content). I proved that the bauxite residue addition had no soil-improving effect in case of all sandy soils.

Applying was not suggested related to metal-contaminated silty (from Nagyhörcsök) soil. Besides a potential advantages (increasing water-holding-capacity, micronutrient supplementation), the risk of pH level’s increasing are important to take into consideration in case of alkalic soils (from Nagyhörcsök and Őrbottyán). Relatively small concentration (max 5%) of material are well useable in case of acidic sandy soil (from Nyírlugos). The pH and the water-holding-capacity were increased by the small concentration in examined, acidic soil.

Kerekes, I., Molnár, M., Feigl, V. (2017): Vörösiszappal kezelt homoktalajok ökotoxikológiai vizsgálata (előadás). 60. Magyar Spektrokémiai Vándorgyűlés és XIII. Környzetvédelmi Analitikai és Technológiai Konferencia, 2017 augusztus 23-25., Debrecen.

Kerekes, I. K., Feigl, V. (2018): Effects of bauxite residue on the avoidance behaviour of *Enchytraeus albidus* (Enchytraeidae). Periodica Polytechnica Chemical Engineering 62(4):415-425.

Kerekes, I. K., Majnovics, Á., Hegyi, D., Molnár, M., Feigl, V. (2018): Vörösiszap ökotoxikológiai hatása homoktalajokban közepes távú mikrokozmosz kísérletben (poster). VIII. Ökotoxikológiai Konferencia, 2018 november 23, Budapest.

Kerekes, I. K., Pusztai, É., Feigl, V., Kemény, S. (2022): Acute ecotoxikological effects of bauxite residue addition on mortality and motion-frequency of *Denrobaena veneta* and *Enchytraeus albidus* (Annelida) in three types od soils. Periodica Polytechnica Chemical Engineering, https://doi.org/10.3311/PPch.19868

II.2. It was my intention that assess the acute lethal and sublethal effects of red mud with using test species (E. albidus) and earthworm (D. veneta).

The effects were depended on the treatment of bauxite residue. Only the powdered form had toxic effect even in case of also the more sensitive test-species, the common potworm *(E. albidus).* Even the higher concentration had no detectable toxicity in suspension or extract.

Only the undiluted bauxite residue samples were toxic to the earthworm species with greater body-size. There is any lethal effect of gypsum treated bauxite residue. The effect of untreated bauxite residue was depended on the concentrations in statistically significant way in case of common potworm (*E. albidus*) test species. The effect was not uniform in the three examined soils, the highest effect was in carbonated sandy soil (OB).

Kerekes, I. K., Feigl, V. (2018): Effects of bauxite residue on the avoidance behaviour of *Enchytraeus albidus* (Enchytraeidae). Periodica Polytechnica Chemical Engineering 62(4): 415-425.

Kerekes, I. K., Majnovics, Á., Hegyi, D., Molnár, M., Feigl, V. (2018): Vörösiszap ökotoxikológiai hatása homoktalajokban közepes távú mikrokozmosz kísérletben (poster). VIII. Ökotoxikológiai Konferencia, 2018 november 23, Budapest.

Kerekes, I. K., Pusztai, É., Feigl, V., Kemény, S. (2022): Acute ecotoxikological effects of bauxite residue addition on mortality and motion-frequency of *Denrobaena veneta* and *Enchytraeus albidus* (Annelida) in three types od soils. Periodica Polytechnica Chemical Engineering, https://doi.org/10.3311/PPch.19868

Related scientific publications

2017 XIII. Környezetvédelmi Analitikai és Technológiai Konferencia (előadás) Kerekes I., Molnár M., Feigl V.: *Vörösiszappal kezelt homoktalajok ökotoxikológiai vizsgálata*

2017 VII. Ökotoxikológiai Konferencia (poster) Kerekes I., Majnovics Á., Hegyi D., Molnár M., Feigl V.: *A vörösiszap ökotoxikológiai hatása homoktalajokban közepes távú mikrokozmosz -kísérletben*

2017 Feigl, V., **Kerekes, I**., Farkas, É., Molnár, M.: *The effect of red mud on thesoil biotas in sandy soil – a microcosm experiment* in: Marilena Buburuzan; Teodosiu, Fava, Gavrilescu, Bertin (ed.) 9th International Conference on Environmental Engineering and Management (ICEEM09): Circular Economy and Environmental Sustainability: Conference Abstracts Book (2017) pp. 283-284., 2 p.

2018 XIV. Kárpát-medencei Környezettudományi Konferencia (presentation) Kerekes I., Molnár M., Feigl V.: *Közönséges televényféreg (Enchytraeus albidus) elkerülési tesztek alkalmazása talajjavító adalékok és szennyezőanyagok minősítésére*

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2019 Tavaszi Szél Konferencia (presentation) Kerekes I., Feigl V., Farkas É., Molnár M.: *Ökotoxikológiai tesztek közönséges televényféreggel bioszén, mint talajjavító adalék hatásának felmérésére*

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2018 Kerekes, I, Feigl, V.: The effect of bauxite residue on the avoidance behavior of *Enchytraeus albidus* (Enchytraeidae)- Periodica Polytechnica Chemical Engineering, 62(4): 415–425.

2020 Farkas É., Feigl V., Gruiz K., Vaszita M., Fekete-Kertész I., Tolner M., **Kerekes I.,** Pusztai É., Kari A., Uzinger N., Rékási M., Kirchkeszner Cs., Molnár M.: Longterm effects of grain husk and paper fibre sludge biochar on acidic and calcareous sandy soils - A scale-up field experiment applying a complex monitoring toolkit- Science of the Total Environment. doi: 10.1016/j.scitotenv.2020.138988. Epub 2020

2022 Kerekes I., Pusztai É., Feigl V., Kemény S.: Acute ecotoxicological effects of bauxite residue addition on mortality and motion-frequency of *Dendrobaena veneta* and *Enchytraeus albidus* (Annelida) in three types of soils- Periodica Polytechnica Chemical Engineering. 66(3), pp. 512–524, 2022. https://doi.org/10.3311/PPch.19868

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