

Supplementary Material

Sequential Extraction Resulted in Similar Fractionation of Ionic Zn, Nano- and Microparticles of ZnO in Acidic and Alkaline Soil

Martin Šebesta, Martin Urík, Marek Kolenčík, Marek Bujdoš and Peter Matúš

1. Artificial rainwater

The batch sorption for contamination of the solid samples alkSoil, acidSoil, and BCR-701 was conducted with a background electrolyte with concentrations of ions typical for the Slovak rainwaters also used in our previous work [2]. The concentrations of mono- and divalent cations and anions were based on the average of five-year measurements (2011-2015) made at five meteorological stations around Slovakia [3]. The prepared thousand fold artificial rainwater concentrate had the chemical composition as follows: 0.2705 g.L⁻¹ of NaCl, 1.2397 g.L⁻¹ of (NH₄)₂SO₄, 0.3044 g.L⁻¹ of NaNO₃, and 1.5096 g.L⁻¹ of Ca(NO₃)₂. Artificial rainwater was created by adding 0.1 mL of artificial rainwater concentrate to 99.9 mL of distilled water.

2. Modified BCR 3-step sequential extraction procedure

After the batch sorption, a modified BCR three-step sequential extraction procedure [29,37,38] was performed on 1 g of solid material (either alkSoil, acidSoil or BCR-701 for control) directly in the 50 mL polypropylene centrifuge tubes (metal-free, sterile, VWR) with polyethylene caps that were used in the amendment part of the experiment:

The first step of the procedure was performed to extract *ion exchangeable* fraction of Zn:

A 40 mL of acetic acid (0.11 mol.L⁻¹) was added to 1 g of the solid materials in the centrifuge tubes. The capped centrifuge tubes were shaken for 16 h (overnight) at room temperature in a rotator at 40 rpm (Multi Bio RS-24, BioSan). The extracts were separated from the solid residue by centrifugation at 3500 g for 20 min and decanted into a polyethylene container and stored in a refrigerator at 4 °C for analysis. Residues were washed with 20 ml of distilled water by shaking them for 20 min at 40 rpm and centrifuging them (3500 g, 20 min). The washings were decanted and discarded.

The second step followed, where a fraction of Zn bound to *reducible* oxyhydroxides of Fe and Mn was extracted:

A 40 mL of freshly prepared hydroxylamine hydrochloride, 0.5 mol.L⁻¹ (solution pH adjusted by addition of 25 mL of 2 mol.L⁻¹ HNO₃ to 1 L volumetric flask to the 34.75 g of dissolved hydroxylamine hydrochloride) in distilled water, was added to the residue from the ion exchangeable step. The extraction procedure was then performed as described above in the ion exchangeable step.

The third extracted Zn from *oxidizable* organic matter and sulfides:

A 10 mL of H₂O₂ (8.8 mol.L⁻¹) was added in small aliquots to the residue from the second extraction step. The centrifugation tube was loosely covered with its cap and digested at room temperature for 1 h with occasional manual shaking. Digestion was continued by heating the vessels to 85°C in a water bath for 1 h, and the volume of H₂O₂ in vessels was reduced to less than 3 mL by further heating of the uncovered centrifuge tubes. A second 10 mL of H₂O₂ was added in small aliquots and the digestion procedure was repeated until all H₂O₂ evaporated and only a moist residue remained. 50 mL of ammonium acetate (1 mol.L⁻¹, adjusted to pH 2 with nitric acid) was added to the cool moist residue. The samples were shaken, centrifuged and the extracts were separated as described in the first, ion exchangeable, step. *Solid residues* were retained for *aqua regia* digestion that has been proposed as a control step in BCR 3-step sequential extraction procedure [37,38] (for details see Supplementary Materials).

The contents of Zn in extracts and *aqua regia* digests were determined by FAAS.

Results from the extractions of uncontaminated lake sediment BCR-701 were compared with certified values, that were provided with the certified reference material, to measure the accuracy of BCR 3-step sequential extraction procedure.

3. *Aqua regia* digestion

Residual material remaining after oxidizable of the BCR sequential extraction was digested after the quantitative transfer of the residual material from polypropylene centrifuge tubes to the glass flasks. The pseudo-total content of Zn was determined by digestion with *aqua regia*, according to the ISO 11466. 1 g of the solid sample was weighed into the reaction vessels, 0.5 to 1.0 mL of distilled water was added to obtain a slurry, then 7.0 mL of 12.0 mol.L⁻¹ HCl followed by 2.3 mL of 15.8 mol.L⁻¹ HNO₃, drop by drop, to reduce foaming, was added. To slowly oxidize the organic matter of the solid samples, the reaction vessels were left to stand for 16 h (overnight) at room temperature. The temperature of the reaction mixtures was then slowly raised until reflux conditions were reached and maintained for 2 h. After cooling the reaction vessel to room temperature, the digests were filtered through Whatman No. 541 filter paper into 50 mL volumetric flasks, the insoluble residues caught by filter paper were washed with a 0.5 mol.L⁻¹ HNO₃ and the volumetric flask was filled with 0.5 mol.L⁻¹ HNO₃ up to the mark.

Reagent blanks contained a negligible amount of contamination and no detectable contamination was found when the sequential extraction reagents were processed and analyzed with the samples (Tables S3 and S4).

4. Analysis of Zn content in solids and liquids

Table S1: Instrumental parameters for determination of analytes by FAAS

| Analyte | Zn |
|-----------------------|-----------|
| Wavelength (nm) | 213.9 |
| Lamp current (mA) | 15 |
| Slit-width (nm) | 0.7 |
| Background correction | deuterium |

5. Results of control experiments

For every solid material, alkSoil, acidSoil, BCR-701, a control experiment without an added form of Zn was completed to observe how much of the natural Zn in the solids materials was released during the consecutive steps of the experiment. The results are average of triplicates with standard deviation. Results can be viewed in Table S2 and Table S3. Relative standard deviation of the sum for alkSoil, acidSoil and BCR-701 was 9.3, 27.7 and 2.5%, respectively. However the standard deviation in absolute numbers was similar for all three solids and the discrepancies arise from lower total concentrations of Zn in alkSoil and acidSoil.

Table S2: Distribution of Zn in the experimental setup of the control experiments with standard deviations

| Sample name | alkSoil | acidSoil | BCR-701 |
|-------------------------|---|---|---|
| Type of sample | Soil CH-cc | Soil CM-st.dy | Lake sediment BCR-701 |
| Steps of the experiment | Zn released [mg.kg ⁻¹ Zn] | Zn released [mg.kg ⁻¹ Zn] | Zn released [mg.kg ⁻¹ Zn] |
| supernatant | 3.31±1.05 | 1.30±0.16 | 9.91±0.50 |
| ion exchangeable | 4.60±0.28 | 4.40±0.65 | 182.05±1.29 |
| reducible | 19.67±1.42 | 5.04±0.40 | 113.94±2.62 |

| | | | |
|---------------|------------|-------------|--------------|
| oxidizable | 2.78±0.49 | 3.54±3.37 | 24.56±1.47 |
| solid residue | 47.48±6.96 | 24.59±10.19 | 128.63±11.18 |
| sum | 77.85±7.21 | 38.87±10.76 | 459.10±11.66 |

Table S3: Comparison of results of the control experiment with certified referential material BCR-701 with the certified values for the BCR-701

| Type of sample | Obtained values | Certified values | Recovery of Zn compared to the certified values |
|-------------------------|--------------------------------------|--------------------------------------|---|
| | Lake sediment BCR-701 | Lake sediment BCR-701 | Zn released compared to the certified values |
| Steps of the experiment | Zn released [mg.kg ⁻¹ Zn] | Zn released [mg.kg ⁻¹ Zn] | Zn released [%] |
| supernatant | 9.91±0.50 | | |
| step 1 | 182.05±1.29 | | |
| supernatant + step 1 | 191.97±1.38 | 205±6 | 93.6 |
| step 2 | 113.94±2.62 | 114±5 | 99.9 |
| step 3 | 24.56±1.47 | 46±4 | 53.4 |
| solid residue | 128.63±11.18 | 95±13 | 135.4 |
| sum | 459.10±11.66 | 460 | 99.8 |

6. Results of the batch experiment and Zn extraction from contaminated solid materials

A batch experiment was conducted on solid materials alkSoil, acidSoil, BCR-701, and concentration of Zn in the supernatants were measured. A BCR sequential extraction was used to examine the sorption of Zn to different soil fraction and after the BCR sequential extraction, the rest of Zn in alkSoil, acidSoil, BCR-701 was extracted by *aqua regia* extraction after (Tables S4-6). Concentration values of Zn from each step of the control experiment were subtracted from the values found in the experiment with contaminated soils. The results are average of triplicates with standard deviation. Relative standard deviation is always bellow 10% in supernatant, ion exchangeable and reducible fractions. Low concentration of extracted Zn in oxidizable fraction meant and increase in relative standard deviation.

Table S4: Distribution of Zn in the system of solid material alkSoil – CH-cc, an alkaline soil

| Zn form | ZnSO ₄ | | ZnO-NP | | ZnO-B | |
|------------------|--------------------------------------|------------------------|--------------------------------------|------------------------|--------------------------------------|------------------------|
| | Zn released [mg.kg ⁻¹ Zn] | Distribution of Zn [%] | Zn released [mg.kg ⁻¹ Zn] | Distribution of Zn [%] | Zn released [mg.kg ⁻¹ Zn] | Distribution of Zn [%] |
| supernatant* | 343±8 | 8.95±0.22 | 921±62 | 28.24±1.91 | 750±62 | 19.07±1.57 |
| ion exchangeable | 2127±12 | 55.56±0.16 | 1421±58 | 43.55±0.89 | 1957±31 | 49.65±0.40 |

| | | | | | | |
|---------------|---------|------------|--------|------------|---------|------------|
| reducible | 1279±29 | 33.40±0.39 | 883±74 | 27.07±1.13 | 1094±26 | 27.79±0.33 |
| oxidizable | 16±1 | 0.41±0.01 | 14±5 | 0.36±0.06 | 13±1 | 0.34±0.01 |
| solid residue | 65 | 1.69 | 23 | 0.71 | 125 | 3.17 |

*Zn not attached to the alkSoil in batch sorption

Table S5: Distribution of Zn in the system of solid material acidSoil – CM-st.dy, an acidic soil

| Zn form Steps of the experiment | ZnSO ₄ | | ZnO-NP | | ZnO-B | |
|---------------------------------------|-----------------------------|-----------------------|-----------------------------|-----------------------|-----------------------------|-----------------------|
| | Zn released | Distribution of Zn | Zn released | Distribution of Zn | Zn released | Distribution of Zn |
| | [mg.kg ⁻¹ Zn] | [%] | [mg.kg ⁻¹ Zn] | [%] | [mg.kg ⁻¹ Zn] | [%] |
| supernatant* | 2702±59 | 65.22±1.43 | 417±10 | 12.30±0.28 | 433±12 | 10.99±0.30 |
| ion exchangeable | 1216±17 | 29.34±0.41 | 2245±22 | 66.26±0.32 | 2754±144 | 69.96±1.83 |
| reducible | 223±6 | 5.38±0.14 | 407±28 | 12.02±0.41 | 515±11 | 13.08±0.14 |
| oxidizable | 3±4 | 0.06±0.10 | 10±3 | 0.30±0.10 | 9±3 | 0.22±0.08 |
| solid residue | <0.005 | <0.005 | 309 | 9.12 | 226 | 5.75 |

*Zn not attached to the acidSoil in batch sorption

Table S6: Distribution of Zn in the system of solid material BCR-701, lake sediment certified reference material

| Zn form Steps of the experiment | ZnSO ₄ | | ZnO-NP | | ZnO-B | |
|---------------------------------------|-----------------------------|-----------------------|-----------------------------|-----------------------|-----------------------------|-----------------------|
| | Zn released | Distribution of Zn | Zn released | Distribution of Zn | Zn released | Distribution of Zn |
| | [mg.kg ⁻¹ Zn] | [%] | [mg.kg ⁻¹ Zn] | [%] | [mg.kg ⁻¹ Zn] | [%] |
| supernatant* | 1120±17 | 26.55±0.40 | 316±4 | 7.50±0.09 | 295±10 | 7.32±0.24 |
| ion exchangeable | 2683±48 | 63.57±1.15 | 3407±58 | 80.78±1.38 | 3271±186 | 81.05±4.61 |
| reducible | 411±8 | 9.73±0.20 | 488±10 | 11.57±0.25 | 463±15 | 11.47±0.38 |
| oxidizable | 6±1 | 0.15±0.01 | 6±2 | 0.15±0.05 | 6±1 | 0.16±0.01 |
| solid residue | <0.005 | <0.005 | <0.005 | <0.005 | <0.005 | <0.005 |

*Zn not attached to the BCR-701 in batch sorption

7. Statistical evaluation of relative Zn concentrations of different Zn forms extracted from the same soil

The relative concentrations of Zn acquired during the three BCR extraction steps (ion exchangeable + reducible + oxidizable = 100%) were compared among the three Zn forms in one soil by single factor ANOVA. An extension Analysis ToolPak in Microsoft Excel (Redmond, WA, USA) was used to find the differences between the means. If a difference was found, ionic Zn, ZnO NP and ZnO B were compared in pairs to find the source of difference by t-Test: Two-Sample Assuming Equal Variances. All tests were done on significance level $\alpha = 0.05$.

When the relative concentrations of Zn extracted by BCR extraction were compared among the different forms, only ion exchangeable fraction in alkSoil had a significant difference in means and the differences were found between ZnO B and ZnSO₄, and also between ZnO B and ZnO NP. The difference in means was minute, around 2% in both comparisons (Table S7).

Table S7: Relative concentrations of Zn extracted by the BCR extraction

| alkSoil | ZnSO₄ | ZnO NP | ZnO B |
|----------------------|-------------------------|-------------------------|-------------------------|
| Ion exchangeable [%] | 62.23±0.52 ^a | 61.41±1.25 ^a | 63.90±0.87 ^b |
| Reducible [%] | 37.41±0.53 ^a | 38.11±1.01 ^a | 35.76±0.86 ^a |
| Oxidizable [%] | 0.36±0.02 ^a | 0.49±0.20 ^a | 0.34±0.01 ^a |
| acidSoil | ZnSO₄ | ZnO NP | ZnO B |
| Ion exchangeable [%] | 84.38±0.02 ^a | 84.39±0.82 ^a | 84.04±1.08 ^a |
| Reducible [%] | 15.47±0.17 ^a | 15.30±0.88 ^a | 15.74±0.97 ^a |
| Oxidizable [%] | 0.22±0.16 ^a | 0.31±0.10 ^a | 0.22±0.11 ^a |
| BCR-701 | ZnSO₄ | ZnO NP | ZnO B |
| Ion exchangeable [%] | 89.90±0.08 ^a | 89.99±0.29 ^a | 90.24±0.15 ^a |
| Reducible [%] | 9.93±0.10 ^a | 9.87±0.31 ^a | 9.62±0.13 ^a |
| Oxidizable [%] | 0.17±0.01 ^a | 0.13±0.04 ^a | 0.14±0.02 ^a |

^a and ^b represent the statistically similar means between different groups at $\alpha = 0.05$ (two-tail t-test).

References:

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