



Analytical methodology

Trace elements in struvite equine enteroliths: Concentration, speciation and influence of diet

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ABSTRACT

Equine enteroliths ~1.5 cm in diameter were collected from an Arabian horse in Louisville, Kentucky, United States. Scanning electron microscopy (SEM) and light microscope imaging of a sectioned enterolith showed two distinct regions of concentric growth outward from the central nidus, a small pebble. After initial growth, acidic colonic fluids permeated the stone inducing recrystallization and alteration of crystals closest to the nidus. A second growth event, when mineral crystallization was again favorable, produced an outer region of unaltered crystals at the rim. The mineral was identified as struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$) by powder X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy. Elemental analysis confirmed concentrations of P, Mg and N consistent with the struvite composition, and detected trace elements Fe ($1050\text{--}1860 \text{ mg kg}^{-1}$), Mn ($262\text{--}280 \text{ mg kg}^{-1}$) and Zn ($197\text{--}238 \text{ mg kg}^{-1}$). All elements were traced to dietary sources, with the Fe:Mn:Zn ratio of the enterolith consistent with that of the horse feed. X-ray absorption fine structure (XAFS) spectroscopy at the Zn K-edge revealed distorted ZnO_4 tetrahedra located between crystallographic planes in the struvite structure forming bidentate linkages to struvite phosphate groups. Emplacement of Zn in structural cavities likely occurs during struvite crystallization. Trace elements and organic impurities increase susceptibility of the enterolith to heat-induced decomposition relative to pure struvite, which could be a consideration for treatment. Results reveal enterolith growth processes, composition and mechanisms of trace metal accumulation that can inform management and prevention of equine enteroliths.

1. Introduction

Equine enteroliths composed of struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$) are a major health issue in California and in the Western United States (U.S.). Removal of large stones ≥ 12 cm that can obstruct or rupture the colon represent more than 25% of colic surgeries at University of California Davis [1]. Reported occurrences of enteroliths in the Eastern United States are rare, in part because the stones are smaller and usually go unnoticed in grass pastures. Some of the major risk factors include diet, for example high magnesium (Mg), nitrogen (N) and phosphorus (P) in feed, genetic predisposition related to breed, high pH of colonic fluids, and mineral content of water supply [1]. The enteroliths grow outward from a central core, or nidus. The nidus is usually a rock fragment, but can be other material, that acts as a nucleation center for crystal growth. As the enterolith grows outward, the crystals form concentric bands similar to those observed in geological mineral concretions. As is common for minerals crystallized from geological fluids, trace elements in waste fluids can accumulate in the mineral during crystallization of

biological concretions. In wastewater treatment, controlled struvite precipitation is used to mitigate the wastewater phosphorus content, and to recover struvite fertilizer [2,3]. In bench-top experiments with real and synthetic wastewaters struvite sorbed trace elements, including zinc (Zn), copper (Cu), arsenic (As) and chromium (Cr), both during and after precipitation [4–7]. Struvite crystallized from colonic fluids also sorbs trace elements, with manganese (Mn), iron (Fe), Zn, Cu and nickel (Ni) detected in equine enteroliths [8,9]. In the current study, enteroliths from a horse in Louisville Kentucky, Eastern U.S. were collected for investigation. The enterolith mineralogy, crystallization process, and trace element content were evaluated, and the role of diet in enterolith formation and composition considered. To better understand trace element sorption to struvite in enteroliths, the speciation and binding mechanism of Zn was determined. The implications of trace impurities for thermal stability, and potential heat treatment of enteroliths was also assessed. Results are of relevance to struvite concretions, including enteroliths and urinary calculi in other organisms that are formed in the presence of, and accumulate trace elements.

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2. Materials and methods

Enteroliths passed by a senior Arabian gelding in a barn near Louisville, Kentucky U.S. were collected. The enteroliths were relatively small with an average diameter of ~ 1.5 cm and shapes that varied from spherical to ellipsoidal. Due to the small size, entire enteroliths were sectioned, and stones minus the nidus were powdered for additional analysis.

2.1. Petrographic analysis

A whole enterolith was selected and a polished thin section prepared for petrographic analysis. The thin section was analyzed with a TESCAN VEGA3 scanning electron microscope coupled with energy-dispersive X-ray spectroscopy (SEM/EDX). The thin section was also viewed under plane-polarized and cross-polarized light using a Zeiss polarizing microscope equipped with a digital camera and image-processing system.

2.2. Mineralogy

The powdered enterolith, minus the nidus, was analyzed by several solid characterization techniques to confirm the sample mineralogy and dominant functional groups. X-ray diffraction (XRD) data were collected from 5 to 60° 2-theta (2θ) in 0.01° increments at a counting rate of 0.3 s per step using a Bruker D8 Advance instrument. Fourier transform infrared (FTIR) analysis was conducted using a Perkin Elmer Spectrum 100 instrument and a universal attenuated total reflectance (ATR) accessory with a ZnSe crystal. Spectra were collected in the 600 – 4000 cm^{-1} range, with a resolution of 4 cm^{-1} . The final spectrum was an average of 20 scans. Results from XRD and ATR-FTIR were compared to a struvite standard (Alfa Aesar, 98%).

2.3. Simultaneous thermal analysis with evolved gas analysis

The thermal properties of the powdered enterolith were determined by simultaneous thermal analysis (STA) coupled with FTIR for evolved gas analysis (EGA). A Netzsch Perseus instrument consisting of a STA 449 coupled to a Bruker Alpha FTIR was used with $\text{N}_2(\text{g})$ as both protective and sample purge. Solids were heated from 25 to 500°C at a rate of $10^\circ\text{C min}^{-1}$ and infrared spectra of evolved gases were collected every 12 s over a range of 600 – 4000 cm^{-1} at 8 cm^{-1} resolution. Results from STA-EGA were compared to a struvite standard, for which data were collected under the same conditions.

2.4. Elemental and organic analysis

Enteroliths were submitted to Midwest Laboratories Inc. for complete elemental analysis by inductively coupled plasma atomic emission spectroscopy (ICP-AES) and nitrogen analysis using a LECO analyzer. Two enteroliths from Kentucky were submitted as whole stones minus nidi. Two samples of California enteroliths, without nidus, from the inner and outer regions of two stones from Hassel et al. [1] (Fig. 1, samples 4 and 6) were also analyzed. The samples were acid-digested by heating in a 6% nitric acid and 1% hydrochloric acid solution. Blanks were prepared similarly, to ensure that background contamination was not of issue. Certified reference material (CRM) standards were used for both the calibration and quality control verification of the instrument. The standards were manufactured according to ISO 9001, ISO 17025, and ISO Guide 34 guidelines and were NIST traceable to standard reference material (SRM) sources. A laboratory control sample (LCS) with a recovery tolerance of $\pm 10\%$ was prepared in parallel with the enterolith samples. The instrument quality control verification checks were considered valid, and thus reported, only if within $\pm 10\%$ of known values. Duplicates were considered acceptable when within 10% of the relative percent difference (RPD). The

powdered enterolith used for XRD and FTIR analysis was acid-digested and analyzed in-house for trace element composition by ICP optical emission spectroscopy (ICP-OES) using an Agilent 5110 instrument. The analysis was run in synchronous vertical dual view (SVDV) mode, and IntelliQuant screening for semi-quantitative determination of elemental composition conducted prior to the complete analysis. The total organic carbon (TOC) concentration of this sample was measured by colorimetric analysis using a TOC direct method reagent test kit and a Hach DR3900 spectrophotometer.

2.5. Extended X-ray absorption fine structure spectroscopy

Elemental analysis detected Zn as a trace element associated with all enterolith samples. The binding environment of Zn in the powdered enterolith sample was determined by extended x-ray absorption fine structure spectroscopy (EXAFS) analysis. Experiments were conducted at beamline 12-BM-B at the Advanced Photon Source (APS), Argonne National Laboratory (ANL), Argonne, IL. The beamline was equipped with a Si(111) monochromator tuned to the Zn K-edge (9659 eV). Data were collected in fluorescence mode using a 13 element Ge detector. To reduce the elastic scattering a Cu filter was placed between the sample and detector. Data reduction and analysis were performed using Athena and Artemis software included with the IFEFFIT program [10].

3. Results and discussion

3.1. Enterolith growth patterns

A section through a typical stone shows the central core or nidus, a chemically altered area surrounding the nidus, and unaltered crystals at the rim (Fig. 1a). Based on EDX analysis, the nidus is a silicate rock fragment composed primarily of Mg, aluminum (Al) and silicon (Si). A thin section through the stone, as viewed with SEM, shows the ring-shaped alteration area composed of struvite grains within a fine-grained matrix (Fig. 1b). A magnified view of the upper left portion of the thin section shows the boundary between the chemically altered region and crystalline struvite (Fig. 1c). The struvite crystals have well-developed cleavage, viewed as small square blocks in the images in the unaltered region. The annular, or ring-shaped area is adjacent to the larger struvite grains, separating the crystalline and altered regions (Fig. 1b, c). This feature is similar to those found in some natural concretions and could indicate dewatering—dehydration during crystallization—at the core of the stone, resulting in volume loss and shrinkage cracks. Acidic colonic fluids could then permeate the stone dissolving struvite. Another growth stage after the stone was sealed and when conditions were favorable for struvite crystallization might explain the unaltered struvite at the rim.

Images taken using a Zeiss light microscope show additional details in the chemically altered region of the enterolith (Fig. 1d, e). This region is permeated by vein-like structures comprised of fine-grained crystals, with small irregular bright spots in the image representing individual crystals (Fig. 1d). The veins may have crystallized from fluids that migrated through cracks in the stone produced by the dewatering process described above. They consist of P and Mg based on qualitative chemical analyses (EDX), indicating these may be composed of struvite. However, the resolution of the SEM/EDX was insufficient to determine the presence or absence of N (also present in struvite). There is optically opaque black material in the struvite crystal that parallels the concentric growth pattern, or fills small radial cracks (Fig. 1d). This material has not been identified but could represent organic matter adsorbed on the struvite surface during crystallization.

3.2. Enterolith mineralogy

The XRD analysis of the powdered enterolith, minus the nidus, shows peaks in the diffraction pattern consistent with a struvite

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