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Discussions

Interactive comment on "The role of microorganisms on the formation of a stalactite in Botovskaya Cave, Siberia – palaeoenvironmental implications" *by* M. Pacton et al.

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This study focuses on the understanding of the origin of a stalactite sampled in an aphotic zone of the cave, and containing calcite and ferromanganese oxides. The debate between biogenic and physicochemical origins of speleothems is an exciting field in constant motion. The emergence of sophisticated tools with high-resolution power allows to constantly bring new insights into this field. To my opinion, the present study, while combining different methods to assess the origin of this stalactite, is suffering from serious issues regarding conclusion linked to the microbial side, as well as straightforward conclusions based on electron microscopy images and microanalysis.

Page 6571, lines 4-6: "The hiatus between layers E and D is the last speleothem surface on which a microbial community was present. Two calcite layers and two hiatuses separate layer B and last period of the microbial activity (. . .)". How can this be stated with no data related to microbiology (e.g. nucleic acid detection)?

The main issue is related to the detection of "biofilms" on mineral surfaces using SEM imaging and EDAX analysis. The organic or mineral nature of features observed under a SEM is still challenging nowadays, and different indirect methods have been used in order to discriminate between both categories (e.g. Pearson et al. 2004). Without organic matter fixation (e.g. glutaraldehyde and/or OsO4), identification of organic material can only be tentative. Moreover, sample preparation into thin sections without a prior step of organic matter fixation and freeze-drying is likely to destroy a large part of the organic material. In Fig 3 (e-f): clays could also lead to the sheath-like feature observed under the SEM (Janssen et al. 2012). Moreover, I am not sure whether the height of the C peak in the EDS spectrum can be used to assess the organic nature of features observed under the SEM. EDAX measurements do not give any information on a molecular level (e.g. type of bond) but only on the elemental composition. Moreover, as stated in the methods section, this is only a semi-quantitative analysis, therefore any stoichiometric conclusion is only putative. In addition to this, although measurements were performed at low energy in order to achieve a small spot-size, there is no certainty that the measure is not also taking into account material underneath the EPS-like structure. Moreover analyses at low energy will not allow a proper detection of heavier elements. I would therefore take those results with more care. In fig. 4c, Si and Mg are detected as associated to the biofilm-like structure, a feature that could indicate that it is more likely a clay-sheath (amorphous clay, as it is not detected using XRD?) rather than EPS. In Fig. 6 (e-f): Regarding the size of the filament pointed by the black arrow, concluding that this is a microbe seems a bit straight-forward to me. Moreover, regarding the spot-size for EDAX measurements (usually about 1μm) and the size of the biofilm-like structure the spectrum most likely is a mixture between the surface-biofilm (if there is any, I must say that it does not appear clear to me) and the

mineral grain supporting it. Fig. 10 (e-f): Similar remark as in fig. 3, identification as EPS is again only tentative here.

The experiments to prove biogenicity of Mn/Fe oxides are not suited to the context of the investigated stalactite. First of all, it is not clear where the biofilm is coming from? Second, if one wants to compare microbial activity in the laboratory to what could have occurred in an aphotic zone of the cave, light should be avoided. Light is indeed an important factor, which will shape the microbial community that will be obtained in the laboratory. Therefore, while Fe/Mn oxides present in the stalactite were formed in an aphotic zone of the cave, minerals produced in the laboratory by microbes were likely produced by phototrophic activity, i.e. most likely by different processes than those occurring in an aphotic environment. In conclusion, I do not see how this allows relating directly the origin of those oxides in the stalactite to a biogenic process (as stated in page 6575, lines 17-18)?

Therefore, the conclusions from page 6579 (lines 26-27)-6580 (lines 1-2) are overstated in comparison to the results obtained. Finally, in page 6580, line 9, in the scenario proposed for the precipitation sequence, it is proposed that cyanobacteria caused the observed microborings. How can cyanobacteria be present in an aphotic environment?

References Pearson VK, Kearsley AT, and Sephton MA. 2004. The in-situ detection of organic material in extraterrestrial samples. Microscopy and Analysis 18, 5-8.

Janssen C, Kanitpanyacharoen W, Wenk H-R, Wirth R, Morales L, Rybacki E, Kienast M, Dresen G. 2012. Journal of Structural Geology 43, 118-127.

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