

## ***Interactive comment on “Can Mg isotopes be used to trace cyanobacteria-mediated magnesium carbonate precipitation in alkaline lakes?” by L. S. Shirokova et al.***

**Anonymous Referee #2**

Received and published: 20 October 2011

### **Main concerns and comments**

This manuscript presents results from a study of bacterially and inorganically mediated hydrated Mg-carbonate formation with a view to observing changing in the Mg isotopic composition through mineral precipitation process.

It would be an important paper, but the presentation and the interpretation of the results appear to be driven by a requirement to demonstrate the results the authors wish to see, rather than what can locally be shown to have occurred in the experiments as reported. My major concerns about this work are listed below.

- 1) The materials and methods are incompletely described, and this made it impossible

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to evaluate the manuscript and to understand what was done. Moreover, results are reported for some experiments that were not described in the Materials and Methods section.

(a) It is impossible to know what the chemical compositions of the starting solutions are. The author should put in a table the starting and final chemical composition of all the experiments assayed as well as the starting and final pH of the solutions. It is not clear which bacteria were used in the culture experiments. In the methodology, authors report that the experiments were made with *Synechococcus* sp., but in the results and discussion sections authors come up with both *Gloeocapsa* sp. and *Synechococcus* sp. experiments, respectively. In the mentioned tables (above), author should also add the name of the bacteria used in each experiment.

(b) The authors present “control experiments” as “abiotic experiments”, which were made by filtering the remaining liquid medium in the culture experiments after mineral precipitation. I want to call the author attention here about how control experiments should be done. First, the medium (solid or liquid) must have the same composition that the medium used for the culture experiments (in the present study named as biotic experiments) are kept under the same physical conditions (P, T, nature of the solution: liquid or solid) as the cultures. Then, you need two controls containing such solution: (a) one without bacteria cells (without inoculating) and (b) another one inoculated with dead/autoclaved cells of the same bacteria you used for the cultures, and not by filtering the solutions of the culture experiments. Authors need to do this for each one of the biotic experiments they present. Further, the authors should name “biotic experiments” as “bacterial culture experiments”. Biotic means everything, any kind of biological stuff. In this case, the authors present pure bacteria culture experiment.

- 2) The authors should present the X-ray patterns of the mineral precipitates in the experiments (nesquehonite, hydromagnesite, dypingite, brucite) and do a more careful and detailed SEM and TEM study. What the authors call needle is an elongated crystal (Fig. 1E). In Fig. 2G what the authors report as hydromagnesite crystals look like an

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agglomeration of mineralized and/or dead cells with rounded particles on their surface but no hydromagnesite crystals. Fig. 2H shows an organic matrix (EPS, organic stuff produced by the bacterium, cells, etc. . .) but no a hydromagnesite crystal. Figures 3b and 3c, do not show any mineral precipitates what the authors identified as hydrous mineral carbonate precipitate is the external envelope of the cell (white colour), but no mineral precipitates. In figures 3a, 3d and 3e; what the authors point out as precipitates could be, but the precipitates then, are located in the EPS and/or organic films produced by this bacterium and no in the cell.

3) Based on the saturation indices of the solutions that the authors report, all the experimental are saturated in hydrous Mg carbonate, nesquehonite,  $SI (\Omega) > 0$  (see my note below). This means that nesquehonite should have precipitated in such solutions inorganically. This leads me to assert that the nesquehonite precipitated in the “biotic experiments” has not been mediated by cyanobacteria (either *Gloeocapsa* sp. or *Synechococcus* sp.), but precipitated inorganically from the solution. In fact, it should be the same precipitation process as the nesquehonite precipitated in the abiotic experiments, that is, inorganic precipitation and no bacterial precipitation. Since the solutions in both “biotic and abiotic experiments” are oversaturated with respect to nesquehonite.

Note: SI is defined by  $SI = \lg (IAP/K_{sp})$ , where IAP is the ion activity product of the dissolved mineral constituents in a solubility product ( $K_{sp}$ ) for the mineral. Thus,  $SI > 0$  implies oversaturation with respect to the mineral, whereas  $SI < 0$  means undersaturation. [Oversaturation: mineral precipitate inorganically from the solution; undersaturation: mineral will not (inorganically) precipitate].

4) Concerning Mg isotopes analyses: (a) Table 3: Rows 4 and 5 (“LIQUIDS”): the  $\delta^{26,25}\text{Mg}$  values in all experimental solutions should be the same because the source of Mg is the same in all the assayed experiments (either water lake, BG-11 medium:  $\text{MgSO}_4 \times 7\text{H}_2\text{O}$  and/or  $\text{MgCl}_2$  powder). The authors must analyse the Mg isotopic composition of the  $\text{MgCl}_2$  powder used in such experiments. Same for the  $\delta^{26,25}\text{Mg}$  values of the solid precipitates, they should have the same value as the precipitating

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solution since these mineral phases have been inorganically mediated and there has not been any microbial or physical (P, T) fractionation. All the solutions assayed in the present study are oversaturated in nesquehonite. The authors should also present the saturation index of hydromagnesite and dypingite. To my knowledge, the value they present for the solid precipitates must be the one of the bacterial cell and bacterial material (EPS, organic films, etc. . .) but not the one of the crystal as they pretend. Furthermore, I am very concern about these values, the authors even go further on this, that they show and assert that there is a Mg fractionation in the abiotic experiments when the Mg isotopic value should be the same in the solution and in the crystal precipitated. Also, authors should know, that isotopes fractionate due to some physical or biological factors, but it is not the case of the experiments presented here (all experiments were kept at the same temperature and not bacteria cells are present in abiotic experiments).

(b) I am very curios to know how the authors separated nesquehonite from dypingite and/or brucite; and the other way around to do their respective Mg isotope analyses.

5) It is impossible to know what mineral phase formed in each experiment. In Table 1 authors report that (1) dypingite and brucite were formed in experiment S-BIO-1, whereas in Table 3 authors say that only brucite was formed in experiment S-Bio-1; (2) no precipitates were formed in experiment S-ABIO-1, whereas in Table 3 authors report that dypingite was formed in S-ABIO-1; (3) in Table 1 authors report that nesquehonite and dypingite were formed in S-ABIO-5, whereas for the same experiment in Table 3 only nesquehonite was formed; in Table 5 authors say that no precipitates were formed in experiment S-BIO-5, whereas in Table 5 the authors report that nesquehonite was formed in experiment S-BIO-5. In table 5 below S-BIO-5, the authors list a new experiment S-f-5 culture: which experiment is this?.

6) Authors should also take into consideration that when you plot and/or compare data, these data must be expressed in the same units (grams, meter, hours...etc). E.g., in Figure 7 authors have plotted the Mg concentration in millimol versus biomass in grams:

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“these two weights should be plotted in the same units, either grams or milligrams!. On the other hand, when authors wish to compare data from biotic experiments with data from abiotic experiments; such experiments must have the same physicochemical conditions (this is not the case of the experiments presented here). In the present work, the experimental solutions should have had the same chemical starting composition and the experiments should have kept at the same temperature. I guess the authors kept all the presented experiments at 21 °C (this is not clear either). The only difference between this experiments should have being that the biotic were inoculated with bacteria cells and the abiotic were not inoculated (without cells).

As the work presented by the authors have a broad lack amongst the methodology, analyses and results, made impossible to know what the authors really did. All these sections seem to be a mixture of different set of experiments and collected data. This needs complete reworking, the authors should rework on these experiments and describe the experimental procedure explicitly. And then, report their results in systematic, clear and coherent ways. Once this is done, the authors should check to be sure their interpretations still hold and they should resubmit the manuscript for review again. Finally, the English should be improved.

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Interactive comment on Biogeosciences Discuss., 8, 6473, 2011.