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Comment on bg-2021-158

Anonymous Referee #2

Referee comment on "Heavy metal uptake of near-shore benthic foraminifera during multi-metal culturing experiments" by Sarina Schmidt et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-158-RC2, 2021

Heavy metal uptake of near-shore benthic foraminifera during multi-element culturing experiments

Authors: Schmidt, S., Hathorne, EC., Schönfeld, J., Garbe-Schönberg D.

Dear Editor,

First of all I would like to apologize for my delay in the review process of the manuscript BG-2021-158 entitled "Heavy metal uptake of near-shore benthic foraminifera during multi-element culturing experiments". The present manuscript present culture experiments of 3 benthic foraminiferal species in a mixed of trace metal solution and study the incorporation of these metal in the newly formed calcite thanks to LA-ICPMS analyses.

The authors performed a very complex and long experiment to study the incorporation of several metals (Cr, Mn, Ni, Cu, Zn, Ag, Cd, Sn, Hg, Pb) in 3 species of coastal foraminifera. I acknowledge to hard work and organisation that was required to perform these kind of experiments. The high quantity of data that came out of these experiments is very interesting but also very complex. The authors try to get the most out of them, maybe too much… It is difficult to get the important messages out of the description and discussion of the data as it is proposed now in the manuscript that is too long. The authors are very thorough in the description of the dataset which is appreciated. But the drawback is that the reader gets lost in the different behaviour of the metal incorporation between species and when considering phase 3 or not…

I have to admit that I am concerned about the interpretations of the data produced, in particular about the partition coefficients. The authors based the DTE calculation either 1) on the slope of a regression line (when available) that is largely based on the data point from phase 3 where the seawater elemental conditions were the least stable, or 2) on single data point calculations where the range of DTE values obtained can be very high (for a given element and a given species). In conclusion, the authors attest that

quantitative reconstructions of seawater elemental concentrations in natural environments could be possible thanks to these partitioning coefficient. Considering the precision of the D determination and the absence of correlation between TE/Ca foram and TE/Ca seawater sometimes, this is out of question to my opinion.

As I was saying the results are really complex, there are no easy and systematic trends observed for a given species or a given element. I understand that the description and interpretation are therefore difficult for the authors. In previous studies, when single element is tested in culture conditions, there was a positive correlation observed between shell and seawater concentration of this element. The absence of such systematic trend in the present study might be an indication that the simultaneous presence of several metals might complicate element incorporation.

I think that the data are anyway very nice to study the difference of incorporation between 2 species from the same genera and between 2 different genera. The authors only shortly discuss this aspect and no figure is focussing on this information, whereas the available data are very useful to look at interspecific variations. The authors could even think about using only data from stable seawater conditions (eg. phase 0-1) to have a closer look at differences in trace metal incorporation (to avoid the addition of uncertainty).

In view of important issues mentioned above and listed below, I would suggest major revision of the manuscript by the author before resubmission to Biogeosciences. I hope that this review will help the authors in this process.

Best regards,

Reviewer 2

Major revisions

- The authors are going too far in their conclusions: in the abstract lines 25-27 "Our calibrations and the calculated partition coefficients… enable the direct quantification of metals in polluted and pristine environments" and in conclusion lines 801-802 "The presented DTE´s allow a direct quantification of metal concentrations in polluted and pristine areas". First, given the really high DTE ranges found in this study (including or not phase 3) and/or DTE (from linear regression) strongly based on the phase 3 data point where the seawater element concentrations are variable, it is not possible to maintain that "quantification" of metal pollution in natural environments is possible. Secondly, the authors are contradicting themselves since they explain in the introduction that a mix of metal may result in interactions that can lead to different incorporation of the metal. Therefore, the mix proposed in this study, which is peculiar since including 10 trace metals at a time (in polluted environment, most often only 1 or 2 metals are above the threshold limit, not 10 at the same time), is not representative of other type of pollutions. The authors should be more measured in their conclusion. The elemental concentration in the shell may definitely be used to look at relative variation of heavy metal concentrations in the seawater through time and space but definitely not to give quantitative data… and only for elements where a positive correlation has been found between TE/Ca foram and seawater.

- As previously mentioned, I think that, instead of describing and discussing each trends or absence of trends observed, they should maybe realise that the absence of systematic tendency (within one element or one species) is unexpected and might be the result of multi-metal experiment since singe metal culture exhibit usually positive correlation between shell and seawater element ratios (cf literature). I would also advise to elaborate more the interspecific differences and maybe on the new elements that have never been measured before.

- To my opinion, there is confusion between the toxicity of metals to the organism and their incorporation into the shell (cf line 27 "This in turn allows monitoring of the ecosystem 27 status of areas"). What are expected from environmental/governmental studies is to evaluate the impact of heavy metal concentrations on the organism life (ability to survive, grow, reproduce…). Here the authors measure the elemental concentration in the shell. The speciation of the metal incorporated in the shell might be different from the one causing toxicity and bioaccumulation in the cell. The elemental concentration in the shell may help to reconstruct variations of seawater elemental concentration but for the moment, the link between this concentration and its effect on marine life is still unknown (and may depends on elements!). I think that the authors should discuss more precisely about this aspect.

- I have problem to understand different aspects regarding the metal mix solution:

- How and where was added this solution? Was it added in the supply tank located on top of the system? In this case, knowing that the pump is flowing at 0.017ml/s, how long would it take to replace and reach the same metal concentration in the culturing vessel (ie Tupperware) as in the tank?
- When was added the solution? According to line 206, we understand that this is added once before each phase. But on line 229-230, it is written "For keeping the metal concentration at the same level over the different culturing phases, water with elevated heavy metal concentrations was fed into the system bi-weekly.". I'm lost, what is this "water with elevated heavy metal concentrations" you are referring to? Is it the stock solution? This need to be clarified…
- Where are taken the samples for trace metal analyses in the seawater? I don't think the information is given (Lines 215-216, line 234 "from both systems")… Are they taken at the outflow of the vessels so that it really corresponds to the concentration of the

seawater in which the foraminifera are growing? Or they were sampled in one of the tank, which would be of course less precise…

- How often was measured the metal concentrations in seawater over the course of the experiment. This should be indicated in the material and method part. For the moment, it is written "frequently" (line 216 and 234) however, when looking at Figure B1, only 1 to 4 data point are available within each phase. This is to my opinion problematic when applying individual curve fit for every phase to calculate the weighted mean value… (see comment after)
- I don't understand the calculation in table 1. The factor between each phase is 10 times but on line 207, it is written "phase 1 = 1 ml, phase 2 = 10 ml, phase 3 = 150 ml". How were calculated these target values?
- The authors used "stock solution" all over the manuscript when referring to the metal mix solution. However, I think it would help to clarify this on line 205 when you first used this term so that it is clear in the discussion (on line 487 and after) that you talk about this stock metal mix solution.

- The culturing system description is very precise but also very long and all the details makes it difficult to understand the general principle. I think that the authors would gain clarity if they explain earlier that a different vessel, freshly filled with calcein labelled forams (if this is correct), is incubated for each different phase of the experiment. At the moment, this essential information appears only (if I'm correct!) on lines 222-223 whereas it should already be said in chapter 2.2.2 or at least beginning of 2.2.4. The description would also be clearer by keeping the same term to describe the same "object".

Abstract

- Line 17: "Seawater analysis… between culturing phases". This sentence is not 100% correct since the increases between phase 0, 1 and 2 are not very obvious for all elements (e.g. Cu, Mn). This is however clear for phase 3.
- Line 24-25: I have the idea that Zn and Cd are showing variations that are more or less similar to other elements (eg. Cd like Pb), no?

Introduction

- There is confusion between "heavy metals" and "trace metals" throughout the manuscript. To my knowledge, the 10 metals studied here are not all considered as "heavy" metals, some are trace metals. I think that this depends on the atomic weight of the element… Please check and use the appropriate terms.
- Lines 69-75: here you talk about the physiological effects. This is interesting but you are looking at the incorporation in the shell which is different (cf comment earlier). The

information is correct but it has to be clear that you will not have a look at this aspect yourself in this study.

- Line 86: "bioavailability". I guess that this is correct to say that if the element is found in the shell, it is bioavailable since it might (depending on the biomineralisation process involved) goes through the cell. However, I would say that this is different from toxicity effect (cf comment earlier).

- Line 43: replace "they" by "the composition of their test"
- Line 46: check in Kotthoff et al. (2017) that Mn/Ca is actually used for O2 or redox reconstructions and not for contamination.
- Line 46: add Guo et al. (2019)
- Line 53: These species are also dominant in intertidal mudflats, not only subtidal areas.
- Lines 61-64: add Barras et al., 2018 for Mn

Material and Methods

- It would be nice to document with SEM pictures and light pictures the 3 species of this study. I think it is even more important knowing that Ammonia and Elphidium are species rising lot of identification discussions! Whatever the name given, it is essential to have to possibility to look at the picture and compare it to literature and also recent DNA papers.
- Lines 118-121: I am wondering if this information is relevant for the manuscript. Since the text is too long, I would suggest to delete this part. Also, the authors mention cores sampled for ecological study which are not presented in the manuscript. This is maybe not necessary?
- Lines 138-150: There are too many details here (eg the size of the petri dish). Some information is repeated several times. For example, the fact that the authors checked several times to be sure that the forams were alive (lines 142-143: "glossy, transparent and undamaged test… cytoplasm present", line147 "structural infill of cytoplasm", line 151 "the color of the cytoplasm was checked"). I don't think the precision of this check at each step is necessary… The important information is that the forams used at the end in the experiment were labelled with calcein and exhibited a green cytoplasm proving that they were active.
- Lines 151-156: I had some difficulties to understand (when I first read the manuscript) when this labelling step happened? Is it only once at the beginning of the entire experiment (before phase 0)? But in this case, the forams added for example at phase 3 could have calcified new chambers in the meantime… Or is it done before each phase in order to add freshly labelled forams in the new introduced vessels? Here the authors should precise this aspect.
- Although the culturing system is well described, it is difficult to not get lost since everything is described with lots of details. Therefore I would recommend to always use the same term when describing one part of it (eg "vessels" for the box containing the well plates, that you should name this way on line 186). On line 195-196, the term chamber is used but we do not really know to what it refers to: well-plate cavities? Vessel? Please try to keep it simple and clear.
- Lines 223-224: One vessel was left from phase 0 to phase 4 (84 days). What was the

interest of this vessel? Were the forams from this vessel analysed? If this is not the case, you should say it to avoid any confusion!

- Lines 286-290 "the total number of chambers was counted before and after the experiment for every specimen (Table 2)": I don't see the interest of counting all the chambers of each foraminifera before and after the phase since the authors used calcein. And this information is not given in Table 2. Moreover, I agree that this is possible to count the total number of chamber in Ammonia species since they are trochospiral. However, this is not the case for Elphidium species since spires of new chambers recover the initial chambers! Therefore, if forams where indeed labelled with calcein just before their introduction into the culture system, I would keep it simple and only mention calcein to identify newly formed chambers.
- Line 312-313: Could you explain why you chose to use NIST612 for calibration and monitoring of instrument drift since the elemental concentrations in this standard are way above the concentrations found in the forams? Moreover, you chose to use a glass standard as quality control whereas it would be more appropriate, to my opinion, to use a carbonate standard with similar matrix to your forams. Moreover, the conditions are similar between carbonate standards and forams (I guess) whereas NIST standards are measured with higher energy and frequency. Please explain.
- Line 334: The authors considered the data as usable if above LOD. However, the limit above which the data can be used for quantitative purposes is commonly defined as the LOQ (limit of quantification). This is defined as 10*SD of the blank. How many data would be excluded from the dataset if the authors use LOQ instead of LOD?
- Lines 364-365: It is not described in the Material and Methods how the living forams were differentiate from the dead ones at the end of each phase. Did the forams lost the colour of the cytoplasm (or their cytoplasm itself) so quickly that you could see it?
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- Line 102: what is Hallig Hooge? Is it still on the field?
- Line 225: Use PSU everywhere or even no unit at all for salinity.
- Line 114: add ", respectively" after "and F.S. Alkor"
- Line 115: "each station" rather than "both stations"?
- Line 119: change "was found" to "were found".
- Figure 2 legend: remove "left" and "right".
- Figure 2a: If I understood properly, there were only 2 vessels per incubator so, to avoid confusion, you should remove 6 of the 8 vessels drawn in figure 2a.
- Figure 2e: this picture is not very clear. Is the shell of the foram entirely fluorescent (ie born in calcein bath)? Otherwise, how many chambers are labelled here? I have the feeling that this is the cytoplasm that exhibit high fluorescence at the bottom since the fluorescence is patchy and fill half of the last chamber...Could you try to show a better picture?
- Line 152: Why did the authors used a concentration of 16mg/L which is different from the recommended concentration given by Bernhard et al. (2006)?
- Line 156: To my opinion, this is not enough time to remove the calcein from the vesicles in the cytoplasm. Anyway, if this seawater is used to calcify 1 new chamber in your experiments, you can hope that this new chamber would exhibit a small fluorescence.
- Line 160: Dagan et al., 2016 is a report. Is it available online somewhere?
- Line 171: it is the air that was filtered?
- Line 171: The authors do not mentioned pH or alkalinity measurements. Did they measure carbonate chemistry during the experiments? At least pH has been measure since it is mentioned in discussion on line 580 "As the pH during the experiment was

stable around 8.0 ± 0.1 (measured twice a week)". This information should arrive in material and methods.
- Line 221: Give the flow rate in ml/min. This would be more adapted.
- Line 225: Please give SD of the data.
- Line 313-314: The authors specify the conditions used to measure glass standards. Could they precise the ones used for carbonate standards?
- Line 323: remove "are expressed"
- Line 331: change "(Rosenthal et al., 1999)" to "Rosenthal et al. (1999)"

Results

- Table 2: In C2 for A. aomoriensis, does it mean that on the 10 forams recovered, 2 were dead but all of them (10) had calcified new chambers?
- Line 368: Since the Ammonia calcified usually more than 4 new chambers, is it possible to see the evolution of seawater metal concentration in the successive chambers of 1 given individual? At least in phase 3? This could help to gain precision in the estimated DTE…
- Figure B1: Could you indicate the error of the measurement on the graph? ON line 340-344, the authors explained that they fit a regression curve on the data to calculate a weighted mean per phase. This seems a good idea when 4 data points are available within a given phase and that a trend can be seen (eg phase 3 for Cr, Ag, Sn). However, this seems difficult when only 2 data points are available and very different (eg Cu) or when the trend is not regular (eg phase 3 for Mn, Ni…). Actually, did you realise that Mn, Ni, Zn and Cd show similar variation though time in phase 3 (lower value at the second sampling time) compared to Cr, Ag, Sn or Pb which show decreasing trends?
- Figure 3: I have the idea that the use of weighted means and standard error of the mean instead of standard deviations, the authors reduce artificially a lot the real elemental variations that they have, mainly in phase 3. Maybe the figure could be completed showing the range of values actually measured in shadow or use box plot to better represent the variability of this artificially created dataset...
- Line 427: remove Cu in "Mn, Ni, Cu, Hg and Sn".
- Figure 4:
  - How are calculated the statistics of the correlations? These correlations should not be based only on the mean values per phase but on the all data set. For example for Ag and Pb, the R^2 and p values are really good but the D is only based on the Phase 3 data which has a high variability! Therefore the D value is not precise and robust.
  - Figure 4 and Table 4: The authors have no objective reasons to fit the correlation through 0 for some elements and not for others. It could be decided on statistical arguments but I have the idea that the authors did not check this.
  - For A. aomoriensis Mn/Ca, there is a problem with the correlation line. This is not possible that the line don't go through the phase 3 datapoint. Please check.
  - The graphs for this figure should have similar y axis range for a given element for the 3 species so that the difference of incorporation between species is highlighted. All graphs should start at 0on the y and x axis. I think that the main (and most robust) output of this study is the difference of incorporation between Ammonia and Elphidium species and this is at the moment only shortly discussed and observable in graphs. This is a shame.
- This is a really good idea that the authors also analysed their data without the data from phase 3. To my opinion, this phase is important to get a trend because the problem when you remove it is that you have no correlation anymore, probably

because the range of seawater elemental concentration is not wide enough. On the other hand, when phase 3 is considered, then a more relevant D value can be calculated but the correlation are only based on this data points and therefore the correlation is not statistically robust.

- Line 435: change "in" to "between"
- Line 459-463 and line 467-468: Repetition compared to what is written in mat and met and in the legend of figure 4 and table. Delete this paragraph.
- All sentences starting with species name, the genera name should be written entirely.
- Line 473: Now authors are removing phase 3 and 2?
- Line 464 to 481: this is very descriptive and difficult to follow…

Discussion

- It is not possible to discuss the significance and meaning of partitioning coefficient that are showing a very high range since this variation is meaningless to my opinion in terms of biomineralisation processes… For example, DCd are varying from values below 1 to values such as 10-20 even 50 in all species (lines 678-679). In terms of incorporation mechanisms, that would mean that some specimens are fractionating against Cd whereas some others (from the same species and in the same condition) would concentrate this element! This is nonsense, right? I would suggest to the author to rather focus on:
  - Elements were a positive correlation is found but instead of using the mean TE/Ca value (eg line 557-559), they should take into account the variability of the data and give a SD for the slope (ie for the DTE). They should also be aware and acknowledge in the manuscript that these correlations are driven by the phase 3 data and might be imprecise.
  - Elements were the range is relatively low so that a general tendency/interpretation might be given.
  - Finally, do not discuss further forward the other elements that exhibit vary wide DTE also if no literature is available on this element, it is interesting to know that this is incorporated and measurable in foraminiferal calcite.
- I have the feeling that the authors use the DTE with or without phase 3 when it helps them to compare with the literature. This is bothering me: is phase 3 really usable to calculate a partitioning coefficient knowing that the seawater concentration of the metal was not stable during this phase and the regression line is totally driven by this single condition?
- Line 506: The authors mentioned the growth of algae as a reason for element concentration changes in the seawater but I understood that the algae were given dead. Therefore, one would not expect algal growth in the experimental set up?
- Line 523-528: this paragraph should be more or less upside down. Since you used calcein prior to the experiment, you do not have to worry that this probe could have

impacted the elemental concentration in your forams. This paragraph could therefore be shorten.

- Line 532: Since most of the references concern culture experiments, I would suggest to add Barras et al., 2018 for Mn.
- Lines 551-552: according to Erez endocytosis biomineralisation, I thought that the composition of the seawater vesicle (ie Mg content) was also modified somehow?
- Lines 559-561: this is interesting but where can we see this information (ie. D vs seawater trace element concentration)?
- Line 559: if D>1, this means that the foram is concentrating the element inside its shell. Therefore, I would not define this as a "non-selective uptake", no?
- Line 561: other studies have observed the same trend of decreasing D with increasing seawater concentrations: Mewes et al. (2015) for Mg and Barras et al. (2018) for Mn.
- Figure 5: The authors refer to this figure for each element but I think that this is also interesting to observe that there is apparently no trend between D and the ionic radius to charge ratio.
- Figure 5: it is strange to me that the author used a single data point for each LogD value. Is it the mean of all measurements? In this case, it would be nice to see the SD since D might be highly variable.
- The authors compare their D values to the literature. Sometimes they compare these values to tropical symbiont-bearing large benthic forams (high Mg content species) or miliolids (line 635, 671, 708) which are known to incorporate much more elements than Ammonia for example and other small benthic foraminifera (low Mg species) (cf van Dijk et al.,2017). This should be specified and discussed.
- Chapter 4.3: as mentioned before I think that Figure 4 should be reworked (or a new figure) in order to observe more easily the differences between species (e.g. similar axis for Ammonia species and different (if needed) for Elphidium). Maybe differences between species would be even better observed when considering only phases where the seawater elemental concentrations are stable?
- Line 724-725: Food is added quite regularly during the experiment. Could the deposition of a layer of food at the surface of the "sediment" could create microenvironments within the hole of the weel-plate? Indeed, the food would be degraded and could influence pH and O2 conditions for exemple…
- Line 727: Read van Dijk et al. (2017) paper but I don't think that the hhigh elemental incorporation of symbiont bearing forams is due to the presence of symbionts but rather to the fact that they are high-Mg content species. Other symbiont barren large benthic forams exhibit high elemental incorporation.
- Line 735-737: be aware that there is a difference between number of chamber added (individual growth rate) and calcification rate (crystal growth rate). Depending on the element, one could expect that slower calcificiation would give more time to remove (or discriminate more against) the element as it is the case for example for Mg.
- Line 738-742: this is an interesting point. I think the authors could potentially unravel this problem if they compare Elphidium data with the first chambers calcified after the calcein stained chamber. Indeed, that would be the forst chamber calcified in the experiment when the seawater elemental concentration was probably the highest. Moreover, as previously mentioned, you could have a look at successive chamber composition to see if you can observe a decreasing elemental composition for the elements exhibiting decreasing trend in seawater.
- Table 5: this table is very interesting and complete but to my opinion, it could be moved in supplementary materials.
- Table 5 : how were the metals analysed in these studies? Analytical techniques used? Extractions? Speciation of the metal?

- Line 488: "…after a while". Please be more specific.
- Line 587: change "possible" to "possibly"
- Line 592: change "effected" to "affected"
- Line 707: this paper from Remmelzwaal refers to post-depositional overprinting. I don't kown this study but are you sure that this DCr corresponds to primary calcite values?
- Line 729: change "Cesborn" to "Cesbron"
- Lines 753-758: too long…

Conclusion

- I think that the authors could highlight the interest to use fossil records (or regular sampling of living forams through time or space) to determine the relative variations of seawater metal concentrations in porewater through time. Although quantitative reconstructions are to my opinion not feasible at the moment, relative variations are usable for elements where a correlation was observed between shell and seawater ratios (not for all elements). The authors should be more realistic in their conclusions.
- The authors could also highlight the interest of forams as they are integrating in their shell the metal concentration over a certain period of time. Indeed, dissolved metal concentrations measured directly in seawater (for monitoring purposes) give the concentration the day of the sampling but this concentration may vary very rapidly… Both aspects should even be mentioned already in the introduction.
- Line 795-796: ok but there is no impact on survival or growth in your experiments.