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Comment on bg-2021-158

Anonymous Referee #1

Referee comment on "Heavy metal uptake of nearshore benthic foraminifera during multi-metal culturing experiments" by Sarina Schmidt et al., Biogeosciences Discuss.,
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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,

The manuscript submitted by Schmidt et al. # bg-2021-158 titled "Heavy metal uptake of near-shore benthic foraminifera during multi-element culturing experiments" is reviewed by me. The authors used live specimens of *Ammonia aomoriensis*, *Ammonia batava* and *Elphidium excavatum* for culture experiments with a mixture of dissolved Cr, Mn, Ni, Cu, Zn, Ag, Cd, Sn, Hg and Pb in artificial seawater. The uptake of these heavy metals in the foraminiferal calcite were measured by laser ablation ICP-MS.

Overall, the manuscript presented good results and discussion. The generated extensive data is highly interesting and is ultimately suited for publication in Biogeosciences, however, the discussion section on 'Application of TE/Ca values in the foraminiferal shell' based on the findings of this study needs to be more clear. See detailed comments below. The conclusion section also needs to present 'quick take away findings' of this study. See below for detailed comments.

For interactive discussions:

1) Higher distribution coefficient values are reported for symbiont-bearing foraminifera species (Line 727). Is it possible or logical to think that lack of food can make the cultured specimens (non-symbiont foraminifera) weak, which can inhibit the incorporation of heavy metals from the surrounding culture medium?

2) Does the different species of Ammonia show similar rates of incorporation (comparison of this study with published culture-studies with other species of Ammonia)?

3) DZn obtained in this study are in good agreement with hyaline species and also a miliolid (Lines 634-636). Some previous work on several miliolid species report elevated concentrations of Zn in their shell compared to the ambient seawater. It would be interesting to discuss about the difference in metal incorporation between miliolid and rotalid species in the discussion section 4.3 Interspecies variability.

Abstract

Line 11: change 'foraminiferal tests' calcite to 'foraminiferal calcite tests'.

From the culture experiments, Pb and Ag are seen to incorporate linearly (Figure 4) in the new calcite of all three foraminiferal species. It would value if the distribution coefficients obtained for these metals are added in the abstract.

Introduction

Line 11: change 'foraminiferal tests calcite' to 'foraminiferal calcite tests'

Line 65: another multi-element culture experiments on large benthic foraminifera *Ammonia hemprichii* reports the proportional enrichment of Mn, Ni and Cd (Sagar et al., 2021) in the foraminiferal tests from the culture solutions, and the thresholds

Line 76: change 'exvacatum' to 'excavatum'

Material and Methods

Line 111: change 'E .excavatum' to 'E. excavatum'

Line 105-106, 116-117, and 124: Were the containers pre-cleaned? If yes, mention the pre-cleaning of the EMSA CLIP and Close boxes used for storing samples from Japsand, and the plastic containers used to collect samples from Kiel Fjord

Line 118-119: Does this apply to all the 9 cores collected from Kiel Fjord? If yes, change the sentence and include the term 'all the 9 cores'

Lines 127-128: Any reason why artificial seawater was used for washing and storage of the samples.

Lines 139-141: Mention the cleaning protocols/steps followed for laboratory ware, which were used to handle the foraminifera specimens

Line 145: 'green colored algae'; mention in Line 107

Line 152: cite reference for calcein (16 mg/l)

Line 154: Were temperature measurements carried at the sampling locations? If yes, mention it in the section 'Field Sampling'

Line 161: Replace 'development' with 'developed'

Line 161: Interchange words 'foraminiferal culturing' to 'culturing foraminifera'

Line 184: Replace 'stable' with 'stably'

Line 218: All salts used were provided in p.a. quality. (does p.a. means pro analysi; please write in full). Please mention the provider of the salts (e.g., SigmaAldrich or CarlRoth or ?)

Lines 200-201: The first, phase 0control phase. No heavyadded. Combine these two sentences.

Line 210: Reference 'Frontallini et al., (2018a)' studies the effect of mercury pollution on cultured benthic foraminifera. Frontallini et al., (2018b) studies the ultrastructural alteration in benthic foraminifera induced by heavy metals (e.g., Pb).

Lines 229-230: Each experimental phase lasted 21 days (three-weeks) and water with heavy metal concentration was fed into the system bi-weekly. Does it mean that in the three-weeks duration, of each phase, the multi-element culture experiment was fed once with the multi-element spike? Please explain clearly.

Lines 256-257: Reword the sentence to 'Metals (Cr, Ag and Sn) were diluted 1/25 and directly introduced into the ICP-MS as they were not retained on the Nobias resin during preconcentration by the SeaFAST system.'

Line 258: add a comma (,) after samples

Lines 260-261. Break the sentence into two. All trace metals except mercury were measured using an Agilent 7500ce quadrupole ICP-MS. Raw intensities were calibrated with mixed standards, which were made from single element solutions covering a wide range.

Line 272: Delete 'from'

Line 271-272: Check the grammar tense of the manuscript; make it uniform (is/was). Example: Line 268: was reduced, Line 271: is nitrogen-purged

Line 283: Replace 'where' with 'were'

Lines 286-287: Reword the sentence "In order to check the growth of foraminifera during the culture experiments, the total number of chambers were counted before and after the experiment for every specimen.

Lines 294-295:removed by rinsing (twice) the sample with Ethanol, which was....

Line 311: ICP-MS/MS (do the authors mean ICP-MS)

Results

Lines 377-378: All values the calculations are based on can be found in the appendix.

Reword the sentence to make it clear.

Line 392-393: In contrast to the text, the figure shows higher Cu/Ca concentrations in the metal experiment of phase 0.

Line 393: The control system in Mn/Ca shows higher concentration in all phases. Reword lines 391-394.

Lines 395-396: Reword the sentence. 'The variation of the metal concentration was highest in phase 3, in both systems, for all elements but Cu, which showed highest variation in phase 0.'

Lines 398-399: For every metal phase experiment, were the samples cultured in the spiked multi-element solution (for that particular phase) for the cultured duration of 21 days. Example: For M2 culture, were the samples spiked with M2-concentration (Table 1) for the whole duration of 21 days? Please see Lines 229-230. Please explain.

Line 401: Change to 'In phase 3, metals Cr, Mn, Cu, Ag and Sn.....

Line 416: Place R2 in brackets.

Line 417: It's also indicated.....or not. Change to 'Cases where regression lines were forced through the origin is indicated.'

Lines 417-419: In cases when a regression.....phases is given. Interchange separately and calculated.

Lines 429-432: In the experiments, there are two systems; Control system (no-spike) and Metal system (spike); Line 374. Each system has 4 phases (0,1,2,3), and phase 0 representing no addition of heavy metals (Lines 200-203). Lines 430-431 says ...when the concentration of these metals in the culturing medium was higher. Please explain.

Line 433: does the phase 3 here belongs to metal system. Add to text.

Line 455: Change 'where' with 'were'

Discussions

Line 487: Fig. B1; Line 830. The TE/Ca values, for most studied metals, are nearly same for phases 0, 1, and 2 of the metal system, although phase 2 is 10x more than phase 1 (Table 1), and 0 being the control phase. Are there any measurements of spiked-seawater (stock and dilutions) before adding to the culture medium? The culture seawater of phases 1 and 2 (in metal system) should show elevated concentrations (in proportions) compared to phase 0, but is not the case. Please explain why?

Lines 493-495:are smaller than expected for phases 0, 1, and 2. Phase 0 is mentioned as the control phase with no addition of heavy metals (Lines 200-201). Then why is the metal concentrations of phase 0 smaller than expected (for normal seawater?). is it also because of reasons mentioned in subsequent lines.

Lines 521-522: Is it possible that the low level of food supplies (as inferred from lack of reproduction) might make the cultured foraminifera specimens weaker and relatively lower amount of metal incorporation in them?

Line 587: change 'possible' with 'possibly'

Lines 615-616: This in turn into the foraminiferal tests. Are the Mn, Zn, and Cu concentrations in the normal seawater (non-polluted) are sufficient as micronutrients considering the fact that these metals are present in the tests of benthic foraminifera recovered from pollution-free environments?

Lines 625-626: Reword the sentence.

Lines 634-636: Titelboim et al., (2018) based on their studies report that miliolid shells might have advantage over hyaline as bio archives, since they record higher values than rotaliids from the same ambient seawater. As mentioned, DZn values of this study are in good agreement with results from hyaline as well as miliolid foraminifera. Please discuss the findings of this study with the findings from Titelboim et al. 2018.

Lines 669-670: change 'which is may connected' to 'which is possibly connected'

Lines 670-676: The mean Pb distribution coefficients obtained from *Amphistegina* spp. by Titelboim et al., (2021) is 12.9. Please add this to your discussion.

Lines 678-679: Please add the results from Sagar et al., 2021b (partition coefficients for Mn, Ni, and Cd from *Amphisorus hemprichii*).

Lines 715-717: Figure 4 shows a positive correlation between the concentrations of Cr in culturing medium and in the foraminiferal calcite of *Elphidium excavatum*. A distribution coefficient of 2.1 has been calculated by the authors for *E. excavatum*. These results are also stated in lines 707-710. The variability of incorporation in *Ammonia* spp. and *E. excavatum* might be because of individual species response. Lines 716-717 are in contrast of the results obtained in this study.

Lines 735-736: When growth is slower, is there a possibility of weak *E. excavatum* specimens and lower incorporation of artificially elevated heavy metals in the culture medium than what they should have done with preferred food source?

Lines 719-742: *Ammonia beccari*, *Ammonia tepida*, have been cultured, by Havach et. al., 2001; Maréchal Abram et al., 2004; De Nooijer et al., 2007; Munsel et al., 2010, for heavy metal partitioning studies. How do their results compare with the findings of this study for *Ammonia batava* and *Ammonia aomoriensis* – a comparison table for the common metals should give a clear picture for *Ammonia* spp. foraminifera. The same can be done for *Elphidium* spp.

Line 745; Table 5: The table is a nice compilation of heavy metal contamination studies in various parts of the globe. The studies referred to in the table have used various natural archives such as water, sediment, bacteria, microalgae, living organisms, and others including benthic foraminifera. A column mentioning the natural archives used by the various researchers is important for the readers. This will help them to not only know the polluted regions of the globe but also give them a quick idea of the archives used for those studies, which might help some researchers to pursue similar studies in the area they live and the best archives available at that place.

The sequence of some authors needs correction in Table 5. For example: Reddy et al., 2005 moves up Williams et al., 2000 for Ni, Zn. Check for other metals too.

Line 749: 'During the past years, many studies were performed to assess the pollution level of seawater.' The natural archives used in the study (for example water, sediments, bacteria, algae, and other should be included in this sentence (See above comment for Table 5).

Line 744: The title of this section is: 'Application of TE/Ca values in foraminiferal shell' – The description in the text talks about the range of concentration used in the culture medium in the current study. The concluding lines of the section (Lines 775-776) says "This means that the concentration range of metals covered by this study is adequate for future research and monitoring of polluted systems". The main point of this research work is to see the incorporation levels of elevated heavy metals in the foraminiferal calcite tests so that they can be used as natural bio archives for monitoring of polluted near-shore marine environments (Abstract Lines 12-13).

This section lacks the description on the results (TE/Ca values in foraminiferal shell) obtained in this study from culture experiments with *A. aomoriensis*, *A. batava* and *E. excavatum* and their application as potential pollution indicators. This section needs modification.

Conclusions

It will be helpful for researchers and readers to pick important findings from this study. Those may be written in point format. For example: '1) All three species showed a strong positive correlation between Pb and Ag in the culture water and their calcite.' The authors should mention the distribution coefficient obtained for these metals from their studies.

Others important findings be written in point format.

Lines 797-799: 'for those elements' – these elements should be mentioned for a quick take away for researchers.

Line 801: 'the presented DTE's' – The DTE's obtained be mentioned here – also which DTE should be chosen, with phase 3 or without, be mentioned in this section.