

## ***Interactive comment on “Heavy metal uptake in foraminiferal calcite: results of multi-element culture experiments” by D. Munsel et al.***

**D. Munsel et al.**

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We appreciate the effort of the reviewer put into our manuscript, which greatly benefited from his/her comments. Each of the comments was addressed separately.

Answers to Anonymous Referee # 1:

Comment 1.1: Perhaps a better experimental design would have been to gauge the effects/artefacts of having a sediment substrate present, given *Ammonia tepida* reproduces copiously in the laboratory even without sediments, as Schnitker et al. demonstrated years ago.

Answer: The experimental design without sediment or any substrate was chosen to observe only changes induced by the applied chemicals. That this may influence the living

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conditions of benthic foraminifers is possible, but other culture experiments with *Ammonia tepida* without sediment (e.g. de Nooijer et al. 2007) showed that experiments without substrate are feasible. The aim of this study was to conduct an experiment under controlled physico-chemical conditions in which the chemistry of the sea water is the relevant parameter. Using sediments would complicate the experiment considerably as water interactions of pore water in the sediment may occur. That there was no reproduction might be due to the fact that the sampling of the foraminifers possibly was carried out after the reproduction already had occurred in nature.

Comment 1.2: At the very least, the authors should note that this species grows easily in the lab, citing appropriate publications.

Answer: In the revised manuscript we noted that this species grows easily in the lab and supported this by accordant literature.

Comment 1.3: The authors assert that the experiments were successful (page 962) yet less than half the specimens grew and reproduction only occurred in one specimen at one (unreported) metal concentration.

Answer: The experiment should be considered successful as no one knew beforehand if any specimen would survive the experiments as there are no published data considering multi-element experiments applied to foraminifera. Yet we decided to rewrite the sentence. We also added the information that the juvenile foraminifer was found in the pool containing the 10-fold concentration.

Comment 1.4: The low reproduction rates probably indicate that conditions were sub-optimal for this species.

Answer: The reviewer is correct! This aspect is explicitly mentioned in the revised version of the manuscript. Nonetheless the foraminifera built new chambers and we could analyse sufficient newly grown calcite.

Comment 1.5: Further, it is highly unlikely that all 400 specimens survived the experi-

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ments, even though the text states this on page 960 line 8.

Answer: Correctly it should read “Nearly all foraminifera survived the culture period of 82 days”. To be more exact, the sentence was rewritten to “Nearly all foraminifera, 95.5% in average, survived the culture period of 82 days – the exact values for each aquarium are listed in Table 2.”.

Comment 1.6: The authors do not note how they determined that all specimens were living. Technically speaking, if the parent reproduces, it did not survive. What do the authors think happened to the offspring of the reproductive event?

Answer: We added the information on how we determined that the specimens were living in section 2.1. The offspring survived the experiment.

Comment 1.7: Ammonia (the chemical species, not the foraminiferal genus) toxicity may have been a problem given the experiment ran for 82 days without aeration and with infrequent water changes. Ammonia was not measured, which is a major flaw in static sediment toxicity studies. Given no juveniles were collected even though they reproduced suggests that toxicity was an issue.

Answer: We do not think that ammonia is an issue in our experiments as we did not use any sediment and the amount of foraminifera as well as the added food is negligible relative to the volume of water in each tank (1.25L). Toxicity though, not induced by ammonia, seems to be an important point.

Comment 1.8: How can the authors confidently present distribution coefficients when [Ni] in calcite of their 20x concentration samples was roughly equivalent to that of the 5x Ni concentration (and similarly for the 10x Cu concentration)? If the authors insist on presenting a distribution coefficient for both metals, then they need to explicitly state it only held for their 1x-10x concentrations.

Answer: The 20x concentration pools for both metals showed very low values in [Ni] and [Cu]. That's why we only showed them on the figures, but did not consider them

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for calculating the distribution coefficients. In the revised manuscript it is stated clearly that only the concentrations 1x-10x were used.

Comment 1.9: The inclusion of discussion about hydrothermal vents is irrelevant, especially since carbonate foraminifera typically do not inhabit vents. If the authors insist on inclusion of this topic, then a better-developed discourse on its relevance and significance needs to be included in the revision, noting that their model species *A. tepida* does not live in hydrothermal vents.

Answer: We certainly do know that foraminifera do not live in hydrothermal vents, but they may live in their surroundings. Doubtlessly though in hydrothermally influenced water trace metal contents are increased and thus hydrothermal influence cannot be ruled out. We clarified that in the revised version of the manuscript.

Comment 1.10: As noted, it is important for the authors to describe how they knew that the foraminifera were living at the beginning and at the end of the experiments. Although it is clear that new chambers were formed in a considerable proportion of the specimens, it is possible that specimens made the chambers early in the experiment and then died.

Answer: We noted above that the technique of determining living foraminifera was added to the revised manuscript.

Comment 1.11: The discussion about antagonism is highly speculative unless the authors did similar experiments varying each metal separately. The authors emphasize that co-varying metals is more realistic yet sediments were not included in the study, which is unrealistic.

Answer: This discussion is speculative and we stated that in the revised manuscript, but it is very sound to with our results of the 20x concentrations. Also the chamber formation in the 20x concentration tank is reduced. To be sure, as the reviewer is saying, similar experiments with variation of each metal separately should be performed. But

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this would go beyond the scope of this paper as this discussion only developed due to the results of the 20x concentrations. We wanted to investigate only the effects of a certain combination of elements – in sediments there are more elements present. It is possible that (more) interactions of the elements occur that cannot be controlled. Even if it may be unrealistic, experiments excluding sediment interactions in a controlled polluted environment should be a first step towards more complex experiments – e.g. experiments containing natural sediments.

Comment 1.12: Furthermore, it is inappropriate to assert that the response of all foraminifera to such metal concentrations will mimic that of *A. tepida*, which is only one of thousands of extant foraminifera.

Answer: Of course each species of foraminifera might and probably will react differently to metal pollutions. But it is necessary to take a first step using one species. We do not intend to assert that our results will apply to all species of shallow water benthic foraminifera species and made this clear in the revised version of our manuscript. Nonetheless trends could be similar in other species.

Comment 1.13: A major shortcoming of the text is that certain passages require clarification. The sentence on page 956 lines 12-14 does not follow from the previous sentence. Also, the sentence requires rewriting since it is impossible to know why foraminifera are “a more realistic” monitor of pollution (more realistic than what?).

Answer: The mentioned sentence indeed causes problems within the context. We rewrote it in the revised manuscript to avoid wrong interpretations and discussions which are not part of this work.

Comment 1.14: How do the authors know that the salinity and pH remained constant during the experiment? This data was not presented, and thus should not be termed “stable” page 957 line 18).

Answer: We know that the salinity and pH remained constant as these parameters

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were measured regularly. In the revised manuscript more details are written in section 2.1. Also a figure (new figure 3) was added to show the stable conditions.

Comment 1.15: How do the authors know that ammonia concentrations did not increase, thereby potentially affecting the foraminifera (see above)?

Answer: We do not know as ammonia was not measured, but it is rather unlikely that ammonia is a major issue as no sediments were involved in the experiments.

Comment 1.16: Minimal detail was provided on replication levels and statistical design. In fact, there was no statistical treatment of the data other than calculating the median of the metal concentrations.

Answer: Replication using synchrotron is not a problem. Replicate measurements of the same spots would lie within 5%. A possible problem rather is the inconsistency of the wall thickness of the chambers. There an error of 10-20% might occur. This is described clearly in Kramar et al. (2010). The data for the distribution coefficient is treated with median and quartiles sufficiently as the data do not show a symmetrical distribution – in fact it is an asymmetrical distribution. For that reason median and quartiles were chosen rather than average and standard deviation. For this data set anything else would falsify the data. Median and quartiles are in our case definitely more reliable (see also Hoaglin et al., 1983; Zhou, 1987). This is all included in the revised manuscript.

Comment 1.17: Some methodological methods are missing, such as were the algae freeze dried or air dried?

Answer: The algae were air dried. This information was added in the revised manuscript.

Comment 1.18: Were the source sediments pristine or polluted? How might the opposite conditions at the source affect results if these experiments were replicated with a different source population?

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Answer: The sediments were pristine. For the opposite conditions, the pre-experiment chambers would have a higher content of trace metals. Depending on the grade of pollution this content may even be higher than in culture grown chambers – depending on the concentrations chosen for the experiments.

Comment 1.19: More precise writing is required regarding the adjusted pH. Page 957 lines 6-7 states the pH was adjusted to “about 8.0”. The term “about” is imprecise.

Answer: The term “about 8.0” was changed to “ $8.0 \pm 0.1$ ”.

Comment 1.20: Feeding seems to have been irregular. Food was offered at the beginning of the ~12-week experiments, after 6 weeks, and after 8 weeks. Text states that food was added “every time the food was depleted”. How was this depletion ascertained?

Answer: Food was given every time it was not visible any more. Then a tip of a spatula of new algae was added. This is also mentioned in the revised manuscript.

Comment 1.21: Please clarify what “up-side down” is for *Ammonia tepida*. Such morphotype has an umbilical and a spiral side, not a top and a bottom.

Answer: The term “up-side down” was exchanged for spiral side.

Comment 1.22: Does the cleaning method employed leach Ca and/or Mg from calcite?

Answer: In the cited literature it is only mentioned that the organic matter is dissolved (e.g. Mashiotta et al., 1999; Pak et al., 2004).

Comment 1.23: Not all foraminifera have calcite shells, as stated on page 958 line 16.

Answer: We meant all the shells of the investigated foraminifera and corrected the sentence to “Due to the fact that the investigated foraminiferal tests consist of calcite, in unfiltered sample spectra the Ni-K lines are overlain by an intense Ca sum-peak”.

Comment 1.24: The tense of the text should be considered. Present tense is awkward

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and atypical in places.

Answer: In the revised version of the manuscript we tried to avoid present tense wherever possible from our point of view.

Comment 1.25: If analytical variance was too large, why didn't the authors extend the analyses (beam time for synchrotron, last ablation raster area)?

Answer: Beam time assigned for synchrotron measurements is very limited. Also the assigned time is limited. For that reason it was not possible to increase the beam time more – otherwise we would have had less data to evaluate and therefore use in the manuscript. The extension of the ablation raster also was not possible – the chamber size is limited and a bigger raster area would have resulted in mix analyses of more than one chamber. The fact that the high space resolved synchrotron measurements and the high integrated LA-ICP-MS measurements are in good accordance to each other consequently indicates that the number of analyses was statistically sufficient.

Comment 1.26: Given that foraminifera precipitate a layer of calcite over existing chambers, the non-fluorescent chambers should also have slightly increased metal concentrations. Did the authors observe this?

Answer: We measured the newly formed chambers and some pre-experiment existing chambers. But due to the fact that foraminifera precipitate a layer of calcite over existing chambers when building a new chamber, the pre-experimental chambers will have a small signal of the culture solution and thus slightly higher concentrations of the trace metals than in the reference pool. But the datasets of newly grown chambers and pre-experiment grown chambers were treated separately and for the calculation of the partition coefficients only the dataset of newly grown chambers was considered.

Comment 1.27: Noting the title for section 3.4, why are Mn and Co data presented in this section?

Answer: The title was changed to “Partition coefficients”.

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Comment 1.28: The authors need to define what they assert is an “acceptable range” (page 962 lines 9-10) and what is “successful” (page 962 line 22). Less than half the specimens grew (although it was asserted they were all living). Not growing a chamber in 12 weeks (in a species that typically lives about a month or so) is not universally agreed to be a success.

Answer: The sentence on page 962 (lines 9-10) was rewritten so the expression “acceptable range” is not present any more. We decided to use different words in the revised manuscript that express the information we want to give more clearly. The last paragraph of page 962 (including line 22) was also rewritten – also see above, where this comment was already answered.

Comment 1.29: Why would a similar behaviour exist for Ni as for Mg, regarding calcein (page 962 line 19)?

Answer: The linear slope of the Ni concentration in the water (see figure 2) suggests no noteworthy effects such as Ni complexation with calcein. To avoid further confusion and doubt we dropped that sentence in the revised version.

Comment 1.30: The statement that malformed chambers were not observed needs to be put into better context with respect to pollution studies.

Answer: We considered this aspect in the revised manuscript.

Comment 1.31: Statistical analyses should be run on these results, especially regarding chamber additions (“slightly lower percentage” page 962 line 24).

Answer: Regarding the results of the chamber additions, we calculated standard deviations according to Poisson distributions and added that information in Table 2. Also a new figure (new figure 4) was designed showing the results of chamber formation during the experiment. The whole last paragraph on page 962 was rewritten for the revised manuscript.

Comment 1.32: Authors need to define “nearly toxic” and what is meant by “resistant”

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(page 964 line 5).

Answer: “Nearly toxic” means harmful and was replaced by “harmful”. This was stated in the revised manuscript and is part of the rewriting of the first part of the first paragraph on page 964.

Comment 1.33: Is it valid to compare present results (lack of sediments, static) with those of le Cadre and Debenay (Was that study static? Did it lack sediments?).

Answer: The study of le Cadre and Debenay (2006) involved some sediment. But as there are only this study and the one from de Nooijer et al. (2007) regarding copper, we originally decided to take it in the discussion. To avoid confusion and doubt about our results, we did not use that study any more to compare our results.

Comment 1.34: The use of colloquialisms is unconventional for scientific literature (“turned out” pg 966 line 17).

Answer: In the revised manuscript the term “turned out to be” was exchanged for “is”.

Comment 1.35: Why aren't Co concentrations presented?

Answer: Measured Co concentrations lay considerably below the detection limit and thus were not presented.

Comment 1.36: The authors present Mn concentrations even though they state they will not discuss this metal (but they do).

Answer: Due to the wide scattering range of Mn we first decided not to discuss this metal. But while writing we noticed that it could be relevant for the discussion (e.g. antagonism) – that's why it was used. In the revised manuscript it is not stated any more that Mn will not be discussed any further.

Comment 1.37: Nickel concentrations are not near the expected concentrations (table 1). Values appearing under x-axes in figure 2 do not correspond to those in table 1.

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Answer: The values below figure 2 represent measurements of natural sea water before the experiments on which basis the element concentrations were calculated. In the figure as well as in table 1 the measurements made during the experiments are shown. Unfortunately there was a typing error in the original data set for the R aquarium with which the calculations were performed. Thus the results from this error propagation displayed are well out of the expected range. This was corrected in the revised manuscript.

Comment 1.38: The authors must clarify why lines and dotted lines in figure 3 are so inconsistent. Specially, why are there two lines in all but one has 3 lines? Why are there a range of 2-4 dotted lines? Why aren't the dotted lines those with the highest and the lowest slope if they bracket the median?

Answer: Figure 3, which is figure 5 in the revised manuscript, has been revised and all the lines and dotted lines are well-defined now.

Comment 1.39: Regarding citations, the lack of references to Chandler's work is inexplicable (e.g., Havach et al., Hintz et al. 2006a in various places). Lack of citations by Alve, who pioneered much of the pollution impact work on foraminifera, is also inexplicable on page 956 line 6. Lack of citing Bernhard et al. (2004) for calcein labelling (page 957 line 15) is also inexplicable. A citation is required for the statement on page 964 lines 14-15.

Answer: All the listed citations are included in the revised version of our manuscript.

Comment 1.40: The title is a bit misleading since only one species of foraminifera was studied. Thus, the title should include the fact that only *Ammonia tepida* was studied.

Answer: The title was changed in an appropriate way.

Comment 1.41: The Abstract is concise yet will need to be updated as appropriate in the context of this review and the resultant manuscript revision.

Answer: The Abstract was adapted to the changes of the revised manuscript.

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Comment 1.42: The manuscript structure is somewhat problematic. Inclusion of discussion points in the Results is inappropriate. For example the sentences on page 960 lines 20-23 are speculation and belong in the Discussion; that on page 961 lines 9-11 also belong in the Discussion.

Answer: All discussing sentences were placed in the Discussion and the Discussion itself was also restructured in the revised manuscript.

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