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

### **Anonymous Referee #1**

Received and published: 31 March 2010

This manuscript exposed a common benthic foraminiferal species to mixed metal enrichments to assess the impact of such exposure to metal concentrations in the foraminiferal calcite. The data presented are novel and of interest to the readership of Biogeosciences yet this manuscript suffers from imprecise misleading writing and poor experimental design. The interpretations and conclusions are only partially supported by the data presented. It is possible that the authors' interpretations and conclusions are warranted although that is unclear given the lack of information on replication, etc.

Perhaps a better experimental design would have been to gauge the effects/artifacts of having a sediment substrate present, given *Ammonia tepida* reproduces copiously in the laboratory even without sediments, as Schnitker et al. demonstrated years ago. At the very least, the authors should note that this species grows easily in the lab, citing

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appropriate publications.

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. The low reproduction rates probably indicate that conditions were suboptimal for this species. Further, it is highly unlikely that all 400 specimens survived the experiments, even though the text states this on page 960 line 8. 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?

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.

How can the authors confidently present distribution coefficients when the [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.

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.

As noted, it is important for the authors to describe how they knew that the foraminifera were living at the beginning of the experiments and at the end of the experiments. Although it is clear that new chambers were formed in a considerable proportion of the

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specimens, it is possible that specimens made the chambers early in the experiment and then died.

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. 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.

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?).

How do the authors know that the salinity and pH remained constant during the experiments? This data was not presented, and thus should not be termed “stable” (page 957 line 18). How do the authors know that ammonia concentrations did not increase, thereby potentially affecting the foraminifera (see above)?

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.

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

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?

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.

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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?

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

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

Not all foraminifera have calcite shells, as is stated on page 958 line 16.

The tense of the text should be considered. Present tense is awkward and atypical in places.

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

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?

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

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 for 12 weeks (in a species that typically lives about a month or so) is not universally agreed to be a success.

Why would a similar behavior exist for Ni as for Mg, regarding calcein (page 962 line 19)?

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

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

Authors need to define “nearly toxic” and what is meant by “resistant” (page 964 line 5).

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?).

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

Why aren’t Co concentrations presented? The authors present Mn concentrations even though they state they will not discuss this metal (but they do). Nickel concentrations are not near the expected concentrations (Table 1). Values appearing under x-axes in Fig 2 do not correspond to those in Table 1. The authors must clarify why lines and dotted lines in Figure 3 are so inconsistent. Specifically, 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 lowest slope if they bracket the median?

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 pg 956 line 6. Lack of citing Bernhard et al. (2004) for the calcein labeling (page 957 line 15) is also inexplicable. A citation is required for the statement on page 964 lines 14-15.

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.

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

The manuscript structure is somewhat problematic. Inclusion of discussion points in

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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 belongs in the Discussion.

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