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Biogeosciences Discussions

Interactive comment on "Bioavailability of organically bound Fe to model phytoplankton of the Southern Ocean" *by* C. S. Hassler and V. Schoemann

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This paper is an interesting examination of organically-bound Fe bioavailability to clonal cultures of environmentally relevant Southern Ocean phytoplankton. All of these experiments touch on important aspects of Fe biogeochemistry in the Southern Ocean HNLC area, and they appear to be carefully conceived, planned and executed. I have quite a few questions and comments about the experimental methods, data analysis and interpretations, especially about some aspects of the methods. In spite of these comments, I think that the paper makes a strong contribution to the literature on Fe uptake and requirements in relation to dissolved Fe speciation.

General major comments

I am particularly interested in the new work they present examining polysaccharide ligands as possible Fe sources to high latitude phytoplankton. Unfortunately, they dont present known conditional Fe-binding constants for the novel carbohydrate ligands they are considering- maybe these have never been measured? I suspect that if available, they would be closer to the putative weaker L2 class than the stronger L1 class. The negligible bioavailability of the siderophore DFB and the organic amine HBED (Fig 2), coupled with the fact that these are the only two ligands they found to be 100% in the $<$0.02 um soluble size class (Fig. 3) is quite intriguing, but is hardly discussed. There is quite a bit of sometimes contradictory evidence on the relative bioavailability of DFB complexes in the literature- see papers by Maldonado, Hutchins, Wells, and Wilhelm. This general subject deserves more attention in the discussion section, as does the relative uptake data from all of the different Fe species they examined.

The authors are to be commended for using recent isolates of algal groups that are actually important in real SO ecosystems, rather than relying on lab weed species routinely available from culture collections. However I see that one of their so-called Southern Ocean isolates, *T. antarctica*, was actually cultured from a Norwegian fjord! Some qualifying text about using this isolate as a model for Southern Ocean strains is needed.

In general , perhaps some consideration of the characteristics of the SO regions where the other three cultures were isolated is also needed, especially when interpreting the uptake results. From the text in the methods section (p. 1682), it appears that two isolates came from relatively nearshore waters near Prydz Bay (Phaeocystis and Chaetoceros), and one came from oceanic subAntarctic waters well north of the Polar Front (Fragilariopsis). Neritic and oceanic phytoplankton have long been known to have very different Fe requirements, see previous work by people like Sunda, Brand and Strzepek- and sure enough, the two coastal isolates also turn out to have typically high coastal Fe:C uptake ratios of 15-20 umol/mol (Fig 4A). The offshore diatom isolate

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is the one with the very low Fe:C ratio of $<$1 umol:mol. This suggests that there might be more than just simple A/V relationships involved in the relative Fe:C ratios of these cultures, and some discussion of this issue of possible habitat-related trends would improve the paper.

The Fe:C uptake ratios of the Fragilariopsis isolate (as low as 0.8 umol:mol from Table 2) seem improbably low to me. Is there really a precedent for such low ratios in SO diatoms in the literature? This also brings up the need to consider the difference between short-term uptake ratios, and cellular ratios for long-term, balanced growth. Measurements of the former do not necessarily constrain the latter. In this particular case, I wonder if the Fragilariopsis cultures were completely healthy and growing normally? The very low intracellular Fe uptake coupled with the very high percentage of extracellular Fe in this isolate (Fig 4) suggest a culture that was possibly not very happy. Fe was scavenging passively onto the cell surfaces, but was not being internalized to any great extent. As I mention below, presentation of growth rate data would help greatly to alleviate these concerns.

Abstract

Page 1678, line 7-8. This wording about the strength of Fe limitation is awkward, and I don't remember reading anywhere in the paper where relative degrees of Fe limited growth were compared. Remove or clarify this text?

Introduction

Page 1679, lines 20-21. There is more involved in competition for Fe between diatoms and cyanobacteria than just uptake mechanisms- cyanobacteria generally have much higher Fe requirements due to their photosynthetic architecture and (sometimes) diazotrophic Fe demands. So I'm not sure it is a given that cyanobacteria will outcompete diatoms just because they might use siderophores.

Just below this- the results of the Trick et al. 1983 paper purporting to show siderophore

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excretion and uptake by eukaryotic phytoplankton have not been reproduced by any other subsequent studies. Charlie Trick himself says he doesn't believe these results any more, and suggested to me that bacteria in the cultures may have been responsible for the observed siderophore production. I would leave this paper out of the background section, it is a bit of a red herring.

Methods

Page 1682- A little text about the growth habit of the Phaeocystis culture is needed. Only in one of the figure legends did I find the information that this strain grew 78% in the unicellular flagellated form. Presumably the other 22% of the cells were in colonies. Obviously Fe uptake characteristics of colonies and unicells are far different, which is briefly mentioned in the section on diffusion limited uptake in the discussion. Maybe this information needs to be put here in a more prominent place than the figure legend. How might your results differ with 100% colonies, or 100% flagellates? And incidentally, how did you count the cells that were in colonies? It is not trivial trying to get accurate microscopic cell counts of colonial Phaeocystis.

Page 1682. Why were Phaeocystis grown at 120 uEinsteins and diatoms at 60? Is there justification for this difference from their photosynthetic parameters? I thought Phaeocystis is believed to be better adapted to lower light levels than diatoms (see Arrigos work on this)? Could this difference in illumination affect biological uptake or medium chemistry in the uptake experiments?

Page 1682. I'd like to see more information on culturing methods and especially on growth rates. The text says cultures were maintained in exponential growth phase, but doesn't give details. Were these batch cultures harvested in exponential phase for the uptake experiments, or were they semi-continuous or continuously diluted cultures? Giving the growth rates for these cultures is also critical- some of the results are potentially consistent with senescent cells that are not growing rapidly (see my comments above). As well, there is usually a strong relationship between Fe-limited growth rates

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and cellular Fe:C ratios (see Bill Sundas and Neil Prices work on this). Presentation of the growth rates of the cultures when they were used for the experiments is really necessary in order to allow interpretation of the results.

Page 1683. I am always a little skeptical about relying on biovolume calculations for normalization of Fe and C uptake , especially in diatoms that have frustules of varying thickness and typically very large cell vacuoles. Although it is certainly important to look at the data this way, it is too bad another impartial normalizer like POC isn't also available for comparison. Next time, consider taking CHN samples too- they are a simple analysis, and can offer another independent way to normalize uptake rates in cells of different sizes.

Page 1684. The experimental design was fairly realistic, since relatively low levels of ligands (15 nM) were added, compared to some past studies. However, since you used SO seawater that had not been UV-oxidized, the natural ligands were presumably also present. This is discussed here in terms of its use as a no ligand added control treatment. The up to 1 nM of strong natural ligands present would also have set up a ligand/ligand competition for the 55Fe bound to the 15 nM added ligand in the other treatments, though. This would seem to be an especially big concern for weaker classes of Fe-binding ligands, as I suspect the saccharides might be. Could some of the uptake assumed to originate from added ligand-bound Fe-55 have come instead from isotope transferred to stronger natural ligands during the one week equilibration time? Have the authors thought about this?

Page 1685. What volume were the uptake experimental bottles, and how much volume was filtered? I tried to calculate how much radioactivity (dpm) there could have been in each filtered sample, but the information I needed to do it isn't here. The reason I wanted to do this is that the molar addition of Fe-55 to the controls seems really low (5% of 0.29 nM ambient Fe is only 0.0145 nM). We have never been able to perform Fe-55 uptake experiments at true tracer levels like that because the specific activity of our isotope is not high enough, you just dont get enough counts in your sample

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to be statistically significant. How many dpms (and associated counting error) did you typically count on a filter? Perhaps you have a source of Fe-55 that is of much higher specific activity than the ones we usually use? Some more specifics about the experimental details, and maybe some statements about average counting errors for the samples (e.g., how low were the Fe55 counts you used in your calculations?), are needed here.

Page 1685. The Tang and Morel 2006 paper presented a nice comparison between the oxalate wash and the Ti wash, and offered some minor recipe modifications and new rinse methods. However, it would be nice to give some credit to the Tovar-Sanchez et al. 2003 work that actually developed the oxalate wash. I will look forward to seeing the Hassler and Schoemann 2009 (still in press?) LOM paper referenced, that from its title apparently has some new insights on surface washing methods.

Page 1686. It is unfortunate that uptake experiments were only done in duplicate. Triplicates would offer much more robust statistics.

Page 1686. I think I understand your reasoning behind calculating Fe:C uptake ratios using a 16 hour 55Fe experiment, and the 14C data from the 2 hour incubation. I still think that it would be much more appropriate to use both values from the 16 hour incubations, though. Arent you most interested in net uptake ratios, anyway? At any rate, since you have the data the least you should do is indicate how Fe:C ratios differ if both are calculated from the 16 hour incubations.

Page 1686. If surface area is the best way to normalize the Fe uptake data, why not show a figure of the data plotted this way instead of burying the numbers in a supplement table? If total cell surface area in the incubation is the dominant single factor affecting Fe uptake (which I'm not sure I agree with, see my comments above), then isn't this factor an over-riding concern for the experimental design? Shouldn't the different species then be compared in incubations with identical surface area/water volume ratios? In your experiments, you did the natural thing and added them at fairly

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arbitrary cell densities, 10,000 mL-1 for the two smaller species and 1000 mL-1 for the two bigger ones. In retrospect, is this the best way to set up the incubation, or should they have been set up based on cell surface area rather than cell number?

Page 1686. There are some interesting things going on with the uptake data that are barely discussed here. For instance, the CAT ligand did nothing to enhance Fe uptake relative to the control for any of the isolates- except for Chaetocerous, which had uptake rates with CAT that were 200% of the control! Chaetocerous and Fragilariopsis both did very well with the PIX ligand too, compared to the all the other groups. There is some text noting this briefly in the discussion, but no possible reasons for these trends are given.

Page 1687. I found it a little distracting that the text discusses Fe partitioning in the cells mostly as Feext:Feint, while most of the figures use % of the total Fe (e.g. Fe-ext/Feext+Feint) to plot the data. I would suggest adopting one convention or the other, and sticking to it.

Page 1689 and Figure 3. Looking at the data, my interpretation would be that all treatments had fairly similar patterns of physical Fe speciation, except the DFB and HBED, which were 100% in the soluble phase. As I said above, this is striking, as is the finding that these were the two ligand complexes that stood out as being least bioavailable to the cells. This needs some consideration- is it a coincidence, or are there some insights to be gained from these results? As for the other minor differences between treatments which are discussed here, this is fine but needs some statistical tests to show significant differences.

Page 1690-1691. The hypothesis that Fe uptake rates are driven by cell surface area while C fixation rates are a function of cell volume can't be the whole story. Simply put, since surface area changes with the square of the radius while volume changes with the cube of the radius, Fe:C ratios would decline exponentially as cells got larger, or increase exponentially for smaller cells. The two larger species of diatoms used here

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had Fe:C ratios that already seem improbably low to me, especially Fragilariopsis, but where does this leave the Fe:C ratios of the many known species that are actually considerably larger? To follow your formula for Fe:C ratios, both the large and small ends of the cell size spectrum would have to have ratios that are out of the realm of possibility. Again, there has to be more determining Fe:C ratios than just cell A/V.

Page 1693. Regarding the conclusion that Fragilariopsis would require long-term, sustained Fe enrichment to bloom, the authors may want to look at the papers from the SOIREE in situ Fe addition experiment in the Southern Ocean, which produced a large bloom of this genus. Apparently, a couple of infusions of Fe over a couple of weeks were enough for Fragilariopsis to respond to the iron in that case.

Page 1694. The text is a little confusing here, and I'm not sure I follow some of the reasoning. Since Chaetoceros and Phaeocystis have the highest Fe:C ratios, how can it be true that they have a lower Fe requirement as compared to the other studied strains (Coale et al 2003, Timmermans et al 2004)? Dont higher Fe:C ratios mean they actually have higher, not lower Fe requirements?

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