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Biogeosciences Discussions

Interactive comment on "Influence of the Amazon River on dissolved and intra-cellular metal concentrations in *Trichodesmium* colonies along the western boundary of the sub-tropical North Atlantic Ocean" *by* A. Tovar-Sanchez and S. A. Sañudo-Wilhelmy

Anonymous Referee #1

Received and published: 21 October 2010

This manuscript presents dissolved metal concentrations and total and "intra-cellular" metal concentrations of Trichodesmium colonies in the sub-tropical North Atlantic near the Amazon River plume. The authors draw conclusions about (1) riverine sources of trace metals to the North Atlantic (2)significance of intra and extracellular metal fractions in the colonies and (3) the involvement of specific metals in different biological processes.

Major Points: In some cases, there is not enough detail included in the methods, regarding both measurements and subsequent calculations, to understand how the authors conducted analyses or to judge the validity of their approaches. There are also some discrepancies in the PCA interpretation that require clarification.

Specific comments: p. 6524 line 12- the term “total metal composition” is vague and unclear. This sentence would benefit from a re-write for clarity.

Note that there are significant populations of bacteria, eukaryotes, and other cyanobacteira associated with Trichodesmium colonies (eg Sheridan et al J. Plankton Research, 2002; Hewson et al 2009 ISME J). I suggest that the authors word the manuscript to acknowledge this and be careful to discuss colonies rather than Trichodesmium. You do this well in some places, but not consistently.

p. 6525 line 10- The flow from the previous sentences to this one is awkward. As written, implies that the Amazon River is a “critical issue” for Trichodesmium. This is not an obvious claim and should be supported, or the sentences should be re-structured.

Methods: In general, I find that there is not enough detail on sampling conditions and trace metal analyses. Specifically:

(1)Was the water from the towed fish sampled, filtered and handled in a trace metal clean environment and how was this environment ensured? The authors should cite and/or describe the “towed fish” for the uninitiated reader.

(2) For the ICP- based dissolved metal analysis, were SAFe standards utilized to ensure measurement quality? This is quickly becoming standard practice in oceanographic trace metal work.

(3) Was dissolved metal extraction efficiency estimated? The Bruland 1985 reference cited for this method does not test the efficacy of this extraction method for all the metals measured in the data shown. The authors should justify its use and efficacy more fully here with additional references.

BGD

7, C3425–C3430, 2010

(4) The authors should provide more details on the mass spectrometry methods and operating conditions employed including specifying use of any internal standards.

(5) The authors should explain how the 100- colony batches from each sampling location were split in two before washing for intra-cellular metal measurement. How was trace metal cleanliness ensured? Was there a known number of colonies per each split sample, and were there enough colonies in each measurement to ensure representative samples for both the intracellular and total metal measurement since these are directly compared? The calculations of extracellular metal:P ratios and direct comparisons between total colony and intracellular metal rest on the quality of the P:col normalization, which is not discussed. Authors should include relevant information required for assessing these normalization choices.

(6) How was chl: col determined? This should be included in the methods.

Results and Discussion:

Dissolved metal data (Figures 2, S1, Table S1): While some of these data look quite clean and show interesting, convincing trends (Mo, Cd, Cu, Fe), others (V, Co) appear very noisy. Given the lack of methodological detail offered and considerable variability in some of these data, I am skeptical of the quality of some of these measurements.

p. 6527 lines 9-14, Fig S1. I don't believe the data for Co warrants this element's inclusion in this statement; the correlation is not tight enough to conclude that the river source here is dominant. Perhaps this conclusion is correct, but these data are too variable to draw it convincingly. For instance, the highest Co value reported here (172 pM) is in one of the highest salinity waters (36, station 4).

p. 6528 lines 10-17. The authors should discuss any evidence as to why the discrepancy for Cu in the riverine endmember calculation may exist as they do for Mo. Authors should also address why there is no evidence of non-conservative behavior in the mixing line in Fig. 2 if there is significant coastal shelf Mo input with a high salinity source.

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7, C3425–C3430, 2010

Also, the last sentence in line 17 does not seem necessary to me. Specific hypotheses could be stated for testing instead.

p. 6529, Ni discussion. Trichodesmium also possesses genes encoding two other Ni enzymes in its genome- urease and a Ni-Fe hydrogenase. The hydrogenase appears to be an uptake hydrogenase which allows for the re-assimilation of energy from H2 produced during N2 fixation in diazatrophic cyanobacteria (eg Seabra et al 2009 FEMS Microbiol. and refs therein). These enzymes and their potential relationship to the biological parameters discussed in this study should be considered.

p. 6529, last paragraph. . It is unclear what the authors mean by "relative abundance of extracellular metal pools" and how this sequence was calculated. This should be clarified.

On the discussion of intracellular metal content in general: quota does not imply requirement necessarily. Metal can be inside a cell without being in biological demand. This can be due to uptake of required metals in excess of the required amount (eg Sunda and Huntsman, 1995, 1997), or it can be due to the co-transport of metals without biological requirements. The authors rightly mention this second case for V, but this concept deserves greater attention throughout the discussion, particularly line 6, p. 6530 as well as in the PCA analyses discussion.

p. 6530 T. weissflogii is misspelled. Check throughout.

p.6530, Fig 3 and 4 The authors should include detailed explanations of how they calculate extracellular metal to P ratios from the measurement of internal metal to P and total metal to P in the text. Since the internal and total measurements are conducted on different sub-samples, I am skeptical of the validity of this calculation as well as all intercomparisons of the intracellular and total colony elements measurements without clarification on the sub-sampling procedure. The authors should clarify, expanding on their response to Dr Twining's comments #1 and 3 and specifically discussing the P:col normalization.

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7, C3425–C3430, 2010

The authors should also add more discussion of trends in the relative ratios of intracellular M:P and total M:P ratios presented in table S1, helping to more fully explain their response to Dr Twining's comment #3.

P 6531 lines 15-20 The authors discussion here seems to imply that cobalamin is involved in C-fixation processes. This should be expanded upon or these lines should be re-worded if that was not the intended implication.

P 6531 and 6532 I am confused about the discussion of the PCA results. I do not have much experience with PCA, but I think the authors seem to be saying that a positive relationship and a negative relationship in PCA mean the same thing. Specifically, they state that a positive relationship between biological DIC drawdown and internal Fe:P and Mn:P ratios supports the involvement of Mn and Fe in related biological processes. Then they also say that a negative relationship between Ni, Mo, and V:P ratios and N fixation rates also suggests biological involvement of these metals in processes related to N fixation. I believe this requires clarification.

I do not believe the authors fully answered the question posed by Dr. Twining in his 4th comment, so I will rephrase, as I had the same question, which is related to the point above. The implication that a negative relationship between N fixation and Ni, Mo, and V:P ratios suggests these metals are important in N fixation seems counter-intuitive. To me, the negative relationship in PCA suggests that colonies fix more nitrogen when they have low Ni, Mo, and V:P ratios. I acknowledge that I do not have much experience with PCA, so perhaps I am confused, but I think clarifying how this relationship implies biological involvement of these metals in processes related to N fixation is needed.

Figure 1: I suggest including some indication of station number or cruise track to help orient the readers.

Figure 2 Authors should indicate where the 24hr six point sampling for the linear regression took place in the figure caption for clarity.

BGD

7, C3425–C3430, 2010

Table S1: I think these data should be included in the main manuscript, which is relatively short compared to others in this journal. I found this table hard to interpret at first, so I suggest some revision of the caption to clarify. The first line should include . . . "total and intracellular particulate metal to P ratios. . ." instead of "total and intracellular metals" Line 5 should start "Elemental" rather than "elements" I think. Also, adding a "d" before the dissolved data would be helpful to clarify the table, ie "P nM" should become "dP nM" and so on.

Figure S2: Where is the regression line for panel F? Again, I think the authors should indicate where the sampling for the regression line occurred in the figure caption for clarity

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Interactive comment on Biogeosciences Discuss., 7, 6523, 2010.