

Interactive comment on “The role of iron species on the competition of two coastal diatoms, *Skeletonema costatum* and *Thalassosira weissflogii*” by S.-X. Li et al.

S.-X. Li et al.

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Dear Editor and Reviewer: Thanks for your suggestions and the reviewer's comments. According to the reviewers' comments (bgd-10-C8670-2014) and your suggestion, we have revised our manuscript thoroughly. Now we resubmit it to you. I hope it could fit your requirement.

Best Regards,

Prof. Dr. Li shun-xing

An itemized response to reviewer's comments is listed as follows:

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Specific Comments:

1. Page 19607, Section 2.1, Lines 1 - 2: How did you test for biological and/or trace element contamination? Be more specific.

Thanks for this good suggestion. We test for biological and/or trace element contamination specific in Section 3.1.

2. Page 19607, Section 2.2: This needs to be rewritten. A simple list of all the instrumentation that was used is not acceptable.

Your good suggestion has been adopted. All the instrumentation that was used has been rewritten as follows:

Agilent ICP-MS (Agilent Technologies, USA) was used to determinate the concentration of trace metals. WHG-102A2-based flow injection hydride generator (John Manufacturing, Beijing, China) was used to measure the N and P concentrations. MK-III-based fiber optic pressure controlled closed microwave digestion system (Shanghai Branch Microwave Digestion Test New Technology Institute, China) was used to microwave digested trace metals test sample. The UV-3200PCS UV-Vis spectrophotometer, provided by Shanghai Spectrum Instruments Co., Ltd. (China) was used to determinate the absorbance of test sample. The double-sided clean bench was purchased from Suzhou Purification Equipment Co., Ltd. (China) and used to alga vaccination. The SPX-300 IC Microcomputer artificial climate chamber (Shanghai Bo Xun Industrial Co., China) was used to culture the algal medium. The Leica DM LB2 microscope Leica (Leica Instruments, Germany) was used to count algal density.

3. Page 19607, Section 2.3, Lines 16 - 17: You should provide a reference, or several, for your FIA methodology.

Thanks for this good suggestion. We have provided several reference for your FIA methodology.

Li, S.X., Liu, F.J., Zheng, F.Y., Zuo, Y.G. and Huang, X.G.: Risk assessment of ni-

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trate and petroleum-derived hydrocarbon addition on *Contricriba weissflogii* biomass, lifetime, and nutritional value. *J. Hazard. Mater.* 268, 199-206, 2014.

Zuo, Y. and Deng, Y.: The near-UV absorption constants for nitrite ion in aqueous solution. *Chemosphere* 36, 181-188, 1998.

4. Page 19607, Section 2.3, Lines 19 - 21: What ICP-MS methodology did you use to measure the Fe concentration? Standard additions? Isotope dilution? Again, you need to be more specific or at least provide a reference.

The ICP-MS operational parameters applied were listed in Table 1 (Shraim et al., 2011).

ICP-MS standard additions were multi-element standards (10 mg L⁻¹, Nos. 2A, USA) and internal standards (including 100 mg L⁻¹ 45Sc, 72Ge, 103Rh, 115In, and 209Bi) were used for trace elements determination (Li et al., 2013).

5. Page 19607, Section 2.3, Lines 1 - 2: How could you accurately measure this Fe concentration if your detection limit was 0.4 nM?

Accuracy and detection limits of iron determination were shown in Section 3.1 as follows:

Iron determination using microwave-assisted digestion and ICP-MS was evaluated by analyzing certified reference materials, including NIES-03 (green algae, *Chlorella Kessleri*) and NASS- 5 (standard seawater). Limit of detection (LOD, calculated as three times of the standard deviation of 3 reagent blank replicates analysed at different time intervals between samples) was 2.84 µg g⁻¹; limit of quantification (calculated as 3.3 times LOD) was 9.37 µg g⁻¹. Found value in NIES-03 and NASS- 5 were 1.82±0.023 mg g⁻¹ and 0.207±0.023 ng g⁻¹, respectively, the results of these analyses in good agreement with certified concentration in both CRMs (1.85±0.092 mg g⁻¹ and NASS- 5 (0.240±0.035 ng g⁻¹). The method described was applicable for the determination of low levels (ng g⁻¹ or µg L⁻¹) of Fe in coastal seawater and marine organisms.

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6. Page 19608, Section 2.4, Lines 11 - 12: Why did you not add trace metals to the culture medium?

We didn't add trace metals to the culture medium was used to ensure that there was no biological or trace metals contamination.

7. Page 19609, Section 2.5, Lines 8 - 9: How were N, P, and Fe concentrations in the medium tracked over the time course of the experiments?

We tested the N, P, and Fe concentrations in the medium daily, and then compensated addition of NaNO₃, Na₂HPO₄, and Fe₂(SO₄)₃ salt to the fixed experiment concentration.

8. Page 19609, Section 2.5, Line 10: What is the distinction between Fe absorption and Fe uptake? Or does Fe uptake represent the sum total of Fe absorption and adsorption?

Fe uptake represents the sum total of Fe absorption and adsorption. Fe absorption (intracellular uptake) and adsorption (cellular surface uptake) were distinguished here because the bioavailability, toxicity, biogeochemical fate, and ecological effects of Fe absorbed by marine algae were quite different from those adsorbed. After absorption (i.e., assimilation) by algal cells, Fe were combined with organic compounds, such as protein and enzyme, and accumulated in the aquatic food chain. In contrast, Fe adsorbed by cellular surface could be partially desorbed into seawater as inorganic metal ions or organic complexes (Li et al., 2013).

Li, S.X., Liu, F.J., Zheng, F.Y., Zuo, Y.G. and Huang, X.G.: Effects of nitrate addition and iron speciation on trace element transfer in coastal food webs under phosphate and iron enrichment. *Chemosphere* 91, 1486- 1494, 2013.

9. Page 19610, Section 2.6, Lines 6 - 8: Why were the filters rinsed with NaOH and HCl?

Because NaOH/ HCl was used to remove any residual inorganic particulate matter on

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the filters (Kannamkumarath et al., 2004; Zhang and Katayama, 2012).

Kannamkumarath, S.S., Wuilloud, R.G. and Caruso, J.A.: Studies of various elements of nutritional and toxicological interest associated with different molecular weight fractions in brazil nuts. *J. Agric. Food Chem.* 52 (19), 5773-5780, 2004.

Zhang, C.F. and Katayama, A.: Humin as an electron mediator for microbial reductive dehalogenation. *Environ. Sci. Technol.* 46 (12), 6575-6583, 2012.

10. Page 19610, Section 2.6, Lines 10 - 11: The equation should be separated from the rest of the text to make it easier to read. needs to be rewritten. You should use the same colors/patterns to represent the Fe fractions of coastal algae A and B in Fig. 3 and 4 to avoid confusion.

Thanks for this good suggestion. The equation has been separated from the rest of the text. The Fe fractions of coastal algae A and B in Fig. 3 and 4 have been used the same colors/patterns to represent.

Technical Comments:

1. Page 19610, Section 2.6, Line 26: Change "0.165 M" to "0.165 nM."

The "0.165 $\mu\text{mol L}^{-1}$ " has been revised as "0.165 nmol L⁻¹".

2. Page 19612, Section 3.2, Line 22: Add "respectively" at the end of the sentence.

The "respectively" has been added at the end of the sentence.

3. Page 19613, Section 3.3, Line 11: Replace *P. donghaiense* with *T. weissflogii*.

The "*P. donghaiense*" has been revised as "*T. weissflogii*".

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/10/C8770/2014/bgd-10-C8770-2014-supplement.pdf>

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Interactive comment on Biogeosciences Discuss., 10, 19603, 2013.

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