<u>RESPONSE TO REVIEWERS</u> on "Particulate trace metal dynamics in response to increased CO_2 and iron availability in a coastal mesocosm experiment" ***by*** **M. Rosario Lorenzo et al.**

<u>Anonymous Referee #1</u>

1)… Generally, the topic of the change of particle trace metal during the marine environment changing such as Ocean acidification (OA) and different Fe availability is very interesting for ocean biogeochemists. I believe the data set is valuable in this field. I feel that, however, authors need to regard more about how they can present their data set to induce conclusion above, which they claimed in conclusion section in this paper. The present contents of this manuscript are not well organized for presenting their data set to conclude the claimed conclusions.

General comments: In construction of this manuscript, "results" section is not constructed only by result, and "discussion" section is not well explained by this study's result (data) ("Discussions" are only like a review of previous knowledge). I recommend that authors should re-construct and re-organized whole part of the manuscript. "Results" section should be used some "Figures" for presenting their data. It makes more easily to understand for readers. "Discussion" section should be related more to data from this study, including which data induce which conclusion more clearly.

In view of both referee comments we have substantially change the Ms attending to their comments. Abstract has been modified, as well as the results and discussion sections. We would like to pinpoint that specifically the Discussion section has been fully re-structured and re-discussed. We believe it is more focused now in order to get a good conclusion. Specific answers to the comments raised follow below.

2)The effect of CO2 did not follow a clear trend in this study, as authors mentioned in the text. The effect of controlled Fe availability by DFB addition/non-addition to phytoplankton bloom is also not clear. How authors induced these their claimed results is not clearly understandable for readers.

This has been modified accordingly in 2.1 Experimental set-up section, Lns 119-124: "*To induce changes in Fe availability, and analyse its effects on the plankton community, 70 nM (final concentration) of the siderophore desferrioxamine B (DFB) (+DFB and −DFB treatments) (Figure 1b) was added to half of the mesocosms on Day 7, when the community was already acclimated to high CO_2. The initial dFe concentration before DFB addition was about 4.5 nM. Even though DFB is a strong Fe-binding organic ligand often used to induce iron limitation in phytoplankton (Wells 1999), DFB additions may also increase the dissolved Fe pool in environments with high concentrations of colloidal and/or particulate Fe, such as fjords (Kuma et al. 1996, Öztürk et al. 2002). By day 17, dissolved iron concentrations were significantly higher (by ~3-fold) in the high CO_2 and DFB treatments than in the control (Segovia et al. 2017). These results support an increase in the solubility of Fe in seawater by either lowering its pH (Millero 1998; Millero et al. 2009) and/or the addition of DFB (Chen et al. 2004).*"

We have added Figure 1, comprising 3 panels referring to CO_2 concentration, dFe and dCu concentrations to better understand the chemical scenario that lead the experiment to the obtained results.

3) For discussion OA influence, I think authors should focus on to show "How particle trace metal concentrations and its ratio changed by CO2 concentrations" by more well presented their own data set.

We have changed the tables by bar plots (now figures 3 and 4) according to reviewer's suggestions and we think it is much clearer now. We have re-written section 3.3-The effects of increased CO_2 and the DFB addition on particulate metal concentrations. Lns 233-242. *"Increased CO_2 and the DFB addition did not significantly affect the concentrations of particulate Al, Ti, Cu, and Pb (Tables 1and S2). Similarly, the addition of DFB did not directly influence particulate concentrations of Fe, but high CO_2 had a significant negative impact on particulate Fe (Tables 1 and S2, Figure 3). Particulate Cd concentrations were also inversely affected by CO_2, but only in the presence of DFB (CO_2; and CO_2 x DFB effect, Tables 1 and S2, Figure 3). All other elements (P, Co, Zn, Mn and Mo) exhibited significant effects by CO_2 and by DFB, but there was also a significant interaction between these two factors (Table 1, S2). This indicates that, for example, particulate Mn, Zn, Mo, Co, and P concentrations were significantly decreased by high CO_2, but only in the +DFB treatments (Figure 3, Table 1, S2,). Similarly, the addition of DFB significantly increased pZn and pMn, but only at ambient CO_2 levels (Figure 3, Table 1, S2)"*

Data are now supported by new Fig 3, Table 1 and Suppl.Table S2.

4) For Fe availability, they need to discuss that "did DFB addition influence positive/negative to Fe availability?". It depends on natural dissolved Fe concentrations.

See answer to point 2 above, and point 5 below.

5) Additionally, authors should show more clearly about relationship between Fe availability and E. huxleyi bloom response, with figure etc. It is very difficult for readers to understand the relation only from the "Tables" number.

We have substituted the Table by a new figure 2 with permission from Segovia et al. Mar. Ecol. Prog. Ser. 2017 for better understanding. This figure shows the temporal development of chlorophyll a (μg L^{-1}) and phytoplankton biomass (μg C L^{-1}) in the mesocosms exposed to different CO_2 and dissolved iron (dFe) treatments. (a) Chlorophyll a, (b) Emiliania huxleyi (5−10 µm), (c) Synechococcus (0.6−2 µm), (d) picoeukaryotes (0.1−2 µm), (e) small nanoeukaryotes (prasinophytes, small haptophytes, 2−7 µm), (f) large nanoeukaryotes (small single-celled diatoms and flagellated forms, 6−20 µm), (g) diatoms (chain-forming Skeletonema sp. 20−> 500 µm), (h) dinoflagellates (20−200 µm).

This is also discussed in a new 4.1 Section: "*The effects of CO_2 and dFe in the plankton community*

In this experiment we investigated changes in particulate trace metal concentrations, in response to increased CO_2 *and/or an addition of the siderophore DFB in a coastal mesocosm experiment. For a better understanding of the processes affecting these stressors, we briefly summarise the mesocosm experiment results originating from Segovia et al. (2017). High* CO_2*, as well as the DFB addition increased dFe concentration. The higher dFe concentrations were sustained in the DFB treatments. A bloom of the coccolithophore Emiliania huxleyi was observed in the ambient* CO_2 *treatments, and was especially massive in the one with the addition of DFB (LC+DFB). On the contrary, the biomass of E. huxleyi was negatively affected by increased* CO_2*. However, increased dFe partially mitigated the negative effect of elevated* CO_2*, indicating that the coccolithophore was able to acclimate better to ocean acidification when Fe availability was high. High dFe also had a positive effect on the cyanobacterium Synechococcus sp, while the rest of the plankton food web did not response to the treatments (Segovia et al. 2017)*"

6) DFB addition inducing more dissolved fraction of TM is artificial response. This is different story from Fe bio-availability. Important for bio-availability is how much free Fe exist under each condition. As one of author well know that DFB-Fe uptake by phytoplankton need very complex mechanisms. Authors should discuss more detail about this part.

This was fully discussed in Segovia et al. 2017 and it is not the aim of this paper which is focused in pMe. However, we suggest to consult Segovia et .2017 for further details. See Lns 423-427. "*Under control conditions at present* CO_2 *concentration (LC, 380 µatm) and no DFB amendment, the globally important coccolithophore Emiliania huxleyi was experiencing Fe limitation (Segovia et al. 2017). The shift between particulate and dissolved Fe promoted a massive bloom of E. huxleyi in the treatments with ambient* CO_2 *due to increased Fe bioavailability (for further details on Fe-bioavailability in E. huxleyi please see Segovia et a. 2017*)".

7)Line 116-117:, 171 Table 2 etc.: Authors described that "The biological and chemical variables analysed were phytoplankton abundance and species composition, dissolved Fe and Cu, nutrient concentration, and particle trace metals concentration". They only show these data in Tables. Figures which present time variation are easier for readers to understand the data variation during the experiments. Please prepare Figures. I can imagine the particle trace metal data was only collected on d12, d17, and d21. But for grasp biological response and chemical environment change, sampling should be done more frequently. If authors have more frequent data for nutrient, cell number for E. huxleyi, etc., it should be plotted to the Figures. Authors described time changing of "diatom" with nutrient concentrations. Authors should make a plot of "day since day0" vs. "diatom cell number", vs. "pigment", vs. "E. huxlei" and vs. "nutrient concentrations" in each mesocosm. It is helpful for reader.

It is indeed done. We have included Chla and phytoplankton biomass in the new Fig.2. Dissolved Fe and Cu in Fig. 1, and Nitrate, Ammonium, Silicate and Phosphate in new Supplemental Figure S1. Additionally reader are encouraged to consult Segovia et al. 2017.

There is no point of re-publishing already published results. Only those that are really essential

8)Line 188-189, 3.2: Authors described "The only metal that showed a time-dependent decrease in its particulate concentrations was Fe. .. .."dMetals", "pMetals". These also should be appeared by figures. 176: Authors described "An increase in dFe was observed in all treatments between day 7 and 17.". In Table 2, there are no data from d7, it should be appeared in the Table 2. And d17 LC-DFB data is decrease. So "all treatments" in this sentence is not correct.

This is already solved by the new figures and the explanation in results section. In addition we have included Supplemental Figure S2 showing the Fe partition coefficients (the molar ratio between particulate and dissolved concentrations) in the different mesocosm treatments; LC: ambient CO_2 (390 µatm); HC: increased CO_2 (900 µatm); -DFB: no DFB addition; +DFB: with a 70 nM DFB addition; on day 12 and day.

9)Line 195-196: Authors indicate that "high CO2 had negative impact on particulate Fe" and "Cd concentrations were also inversely affected by CO2". These parts are difficult to understand which data indicate this fact. Line 197-198: Authors described "All other elements (P, Co. . .. .these two factors was clear (Table 5)". Please explain how clear as like Cd description in previous sentence. Only showing Table 5 is not enough explanation to reader.

We have rewritten 3.3-The effects of increased CO_2 and the DFB addition on particulate metal concentrations as follows: "*Increased CO_2 and the DFB addition did not significantly affect the concentrations of particulate Al, Ti, Cu, and Pb (Tables 1and S2). Similarly, the addition of DFB did not directly influence particulate concentrations of Fe, but high CO_2 had a significant negative impact on particulate Fe (Tables 1 and S2, Figure 3). Particulate Cd concentrations were also inversely affected by CO_2, but only in the presence of DFB (CO_2; and CO_2 x DFB effect, Tables 1 and S2, Figure 3). All other elements (P, Co, Zn, Mn and Mo) exhibited significant effects by CO_2 and by DFB, but there was also a significant interaction between these two factors (Table 1, S2). This indicates that, for example, particulate Mn, Zn, Mo, Co, and P concentrations were significantly decreased by high CO_2, but only in the +DFB treatments (Figure 3, Table 1, S2,). Similarly, the addition of DFB significantly increased pZn and pMn, but only at ambient CO_2 levels (Figure 3, Table 1, S2)*"

We believe is much clearer now. Note that Table 5 has been substituted by figure 3 to make it easier and that Table 1 and S2 also support these data.

10)For "3. Results" section, all subtitle is not well organized. Some contents can be compiled to one (For example, 3.1 and 3.2 can merge for "biological chemical response in mesocosms". And particle trace metal variation in different treatment in 3.2, 3.3, and 3.4 can merge to one section.

Some of he sub-headings have reorganised. We have accepted the suggestion of "*3.1 biological chemical response in mesocosms*". However, we have not merged the following sections as we think it will be rather confusing to the reader.

11)Title and contents of subsection in 3.6 and 3.7 are part of "discussion".

These results parts have been re-structured and part of the text has been moved to Discussion section.

12) Discussion Line 247-248: "Our results demonstrate that in the studied fjord, particulate Ti and Fe concentrations were dominated by lithogenic material.". Authors need explanation how they judged this. The explanation is appeared in section 3.6 result (actually this is discussion). Please indicate clearly "this data is shown in Figure 1". Line248-250: "In contrast, particulate Cu, Co, Mn, Zn, Mo and Cd concentrations were correlated with P concentrations, as well as phytoplankton biomass, suggesting strong biogenic influence on their distribution (Table 6)". Authors need explanation how they judged this. The explanation is appeared in section 3.7 result (actually this is discussion). Please indicate clearly "this data is shown in Figure 1". Only showing Table 6 is not kind for reader. This part is overlapped to 4.2 section. It should be in to 4.2 section with detailed explanations with Tables and figures.

This one of the parts that have changed most. This section is supported by Fig 5 and Table 4. Thus, we have fully restructured and re-discussed 4.2 Particulate Fe and Ti are associated with lithogenic sources, while particulate Co, Cu, Zn, Cd, Mo and Mn are associated with biogenic sources, as follows, Lns 265-326:

"The particulate trace metal concentrations (nM, mean of all treatments and dates) during the experiment were highest for Al, Fe and Zn, and lowest for Co and Cd, following this trend: Al ≈ Fe ≈ Zn > Ti > Cu ≈ Mn > Mo ≈ Pb > Co > Cd. Lithogenic particles are enriched in Al and low in P (average crustal Al and P content is 2.9 mmol Al and 0.034 mmol P g^{-1} dry weight, Taylor 1964), while biogenic particles are enriched in P and low in Al (average plankton Al and P content is 0.001 mmol Al and 0.26 mmol P g^{-1} dry weight, Bruland et al. 1991). Therefore, the distinct high abundance of Al and P in lithogenic and biogenic particles, respectively, can be used to evaluate the relative contribution of lithogenic and biogenic material in our particulate samples. In order to do this, first, it is important to establish that the vast majority of the measured particulate P is associated the biogenic fraction. In this study, the abiotic P was estimated using particulate Al concentrations (nM) and the P:Al ratio in crustal material, and was calculated to be negligible (< 1% of the total measured particulate P). In addition, a significant correlation ($p < 0.003$) was found between particulate P concentrations and phytoplankton biomass (Table 4). Therefore, we assume a constant trace metal composition in biogenic particles (assuming they are rich in phytoplankton) and lithogenic particles (assuming they are rich in crustal material). We then calculated the expected metal concentrations in the particulate samples assuming that all the P measured in the particles is associated with a biogenic fraction, and that all Al in the particles is associated with the lithogenic fraction. Thus, for a given trace metal, its expected particulate trace metal concentration in seawater (mol L^{-1}) can be calculated as the sum of the contribution from biogenic and lithogenic particles, so that:

$$[Me] = a\,[P] + b\,[Al]$$

where [Me] is the total concentration of the metal (mol L^{-1}) expected in the particulate sample; [P] is the P concentration measured in the particles (mol L^{-1}); [Al] is the Al concentration measured in the particles (nM L^{-1}); a is the average, well-known metal content in biogenic particles, normalized to P (i.e. mol Me: mol P in marine plankton) and b is the average, well known metal content in lithogenic particles, normalized to Al (mol Me: mol Al in the Earth crust). For example, on day 21 in the HC-DFB treatment, the concentrations of particulate Al and P were 8.22 and 134.8 nM, respectively (Table S2). Assuming a constant 0.0051 mol Fe: mol P in biogenic particles and 0.331 mol Fe: mol Al in lithogenic particles (Table 2), we calculated an expected particulate Fe concentration of 3.41 nM, where 80% was associated with lithogenic material and 20% with biogenic material. Similar calculations were made for the bioactive metals Mn, Co, Cu, Zn, Cd, and Mo (Table 5). Our calculations indicate that on average, particulate Fe was dominated by the lithogenic component (accounting for an average of 78% of the total expected particulate Fe), while for particulate Co, Cu, Zn, Cd, and Mo the biogenic fraction dominated (accounting for 94, 95, 99, 94 and 98%, respectively, of the total expected concentration; Table 5). Particulate concentrations of Mn were also dominated by the biogenic fraction (65% of the total), but the lithogenic fraction was also significant (35%). Moreover, the expected particulate Mn and Fe concentrations closely matched the particulate Mn and Fe concentration we measured (accounting for an average of ~ 71% of the measured Mn, and 115% of the measured Fe). For other metals (i.e. Cu, Mo and Zn), the expected particulate concentrations (nM) were lower than measured (23% of the measured pCu, and 8% of measured pZn; Table 5). This suggests that the particles were enriched in Cu, Mo, and Zn relative to what is expected based on natural marine plankton metal quotas (Bruland et al. 1991) and crustal ratios (Taylor 1964).

To further establish the lithogenic or biogenic source of the pMe in the particles, the particulate metal concentrations were normalized to the concentrations of particulate P and Al (Figure 4, and Table 2). These ratios were then compared with well-known molar ratios of metal to Al in the crust (Taylor 1964) and of metal to P ratios in marine plankton samples (Ho 2006) and cultures (Ho et al. 2003) (Table 2). The average Fe: Al (506 mmol Fe: mol Al) and Ti:Al ratios (119 mmol Ti: mol Al, Table 2) were relatively similar to crustal molar ratios (331 mmol Fe: mol Al and 39 mmol Ti: mol Al; Taylor 1964). Additional evidence for the significant lithogenic component in particulate Ti and Fe was gathered from Figure 5, where we plotted the molar ratios of the metals relative to P in the collected particles against the Al:P ratios measured in those same particles. The slope of these data [(Fe:P)/(Al:P) = mol Me: mol Al] is the ratio of Me:Al in the particles and can be compared to well-known Me:Al crustal ratio. Visually, if the data nicely fit the Me:Al line for crustal material, these metals are mainly associated with the lithogenic component, as evident for Fe and Ti (Figure 5). These combined results suggest that in our experiment, particulate Fe and Ti concentrations were enriched by lithogenic material. In support of this finding, we also found no significant correlation between particulate Fe and Ti concentrations (nM) and either the total plankton (phytoplankton and microzooplankton) or E. huxleyi biomass (µg C L^{-1}; Table 4).

In contrast, when the P-normalized metal ratios in the particles collected from the mesoscosms were plotted against the Al:P ratios in these particles, there were no correlations for the following metals Co, Cu, Zn, Cd, Mn and Mo (Figure 5), indicating *that these particulate metals were not enriched in lithogenic material. Our measured metal: P ratios were comparable to plankton ratios in natural samples and in cultures (Table 5). The concentrations (mol L^{-1}) of these metals (i.e. Cu, Co, Zn, Cd, Mn, Mo), as well as P, also showed significant correlations with the biomass (µgC L^{-1}) of E. huxleyi and that of total plankton cells ($p < 0.05$, Table 4), supporting a significant influence of the phytoplankton in the distribution of these particulate elements.*

13)Line 251-252: "Changes in CO2 and/or Fe levels affected total particulate and biogenic metal concentrations for some metals.". This part of results is not well presented in manuscript overall. Authors should regard to present some figures which can compare particle and biogenic metals concentrations among each treatment.

We have included Figure 3 and 4, and eliminated the corresponding tables that were unclear.

14)Line 255-263, 4.1 Efficacy of the oxalate-EDTA wash removing lithogenic trace metals from particles: First half part of this section is should be move to "results". Especially from line 260-263, "In general, the concentrations of Fe and Co in the particles were decreased the least by the oxalate wash (by ~ 25%), while Mo and Pb concentrations were decreased the most (by ~70%). The concentrations of particulate Cu, Zn, Cd and Mn were reduced by 50% by the oxalate wash. As shown previously (Sanudo-Wilhelmy et al. 2004), the oxalate reagent also removed extracellular P (by ~20%).".

This has been moved to material and methods and now reads as follows. Lns 176-195:

"2.3.3 The effect of oxalate-EDTA wash on particulate trace metal concentrations

To better estimate the biogenic fraction of the particulate metals, the filters were washed with an oxalate-EDTA solution, which removes extracellular metals and oxyhydroxides (Tovar-Sanchez et al., 2003; Tang and Morel, 2006). In our study, the oxalate wash significantly decreased the concentration of all particulate metals, with the exception of Al and Ti (Tables S2 and S3), as observed by Rauschenberg and Twining (2015). The quantity of metal remaining after the oxalate wash (i.e. biogenic fraction) varied among elements (Tables S2 and S3). In general, the concentrations of Fe and Co in the particles were decreased the least by the oxalate wash by ~ 25%, while Mo and Pb concentrations were decreased the most by ~70%. The concentrations of particulate Cu, Zn, Cd and Mn were reduced by 50% by the oxalate wash. As shown previously (Sanudo-Wilhelmy et al. 2004), the oxalate reagent also removed extracellular P (by ~20%, Table S2 & S3). Compared to Rauschenberg and Twining (2015), the estimates of the biogenic fraction, after the oxalate wash, were in agreement for Co, Cu and P, and lower for Fe, Mn, Zn and Cd concentrations.

However, the efficacy of the oxalate wash to dissolve Fe, and other metals, from lithogenic particles is not well constrained (Frew et al. 2006, Rauschenberg and Twining, 2015, King et al., 2012). Therefore, the results obtained after the oxalate-EDTA wash should be interpreted with caution because we do not know whether the removed metal fraction is a) only lithogenic; b) mainly lithogenic but some biogenic fraction is also removed, or c) *whether metals absorbed onto particles are equally labile to the wash on biogenic and lithogenic particles. Given that many of the trends we observed were identical for the oxalate-EDTA washed and non-washed particles [i.e. higher Me concentrations in the LC+DFB treatments (Table S2 & S3) and positive correlations between phytoplankton biomass and Me concentrations (Lorenzo-Garrido 2016)], below we present and discuss only the non-oxalate wash results"*

So that, oxalate-wash is not further discussed. Yet, it is important to maintain this paragraph in the Ms.

15) Line 282:" Me:P ratios we measured in the particles are similar to those of natural phytoplankton assemblages (Ho, 2006) and of Emiliania huxleyi cultures (Ho et al., 2003).". If authors want to compare their filed data to previous reported data by Ho, 2006, and Ho et al., 2003, authors should show the previous study's number with their data on to Tables or Figures with citation. Otherwise, authors just state "similar" to natural plankton but did not show any evidence.

This has all been re-organised and re-discussed as required by both reviewers. Please see point 12 above.

16) Line 311-312, 319-320: "Interestingly, we also found a putative ZIP-transporter gene. ZIP-transporters are. . .. ., such as tRNA synthetase, reverse transcriptase, metallo-carboxypeptidase, ABC-Zn-transporter and CDF-Zn-transporter..". If authors want to say "we found", they should show their data and discuss with using their data. If this "gene part" is part of other study, they should cite the other study appropriately. This discussion section is very strange for this aspect. It is written like author's original data for this study.

We have removed the paragraph relating to referee comments. We fully agree with the comment.

17) Line 329- : Discussion on Cu:P should construct by using their data, what their data's characteristics, what their data indicate, what is authors claim from the data, which previous knowledge supports their claims. This section 4.2 is like just a review of other papers.

We have re-written this part and better discussed our own data as follows, Lns 352-362:

Similarly, the Cu:P ratios in the collected particles were relatively elevated (1.4 ± 0.8 mmol Cu: mol P) compared to those of other phytoplankton, including E. huxleyi (Table 2). The dissolved (7.7±0.41 nM Cu, Figure 1) and particulate Cu concentrations (0.35±0.25 nM, Table S2) in our experiment were high, and similar to those previously measured in this fjord (Muller et al., 2005). Rain events (or wet deposition of anthropogenic aerosols) in this fjord result in high dissolved Cu and the active production of strong organic ligands by Synechococcus—to lower the free Cu concentrations (Muller et al., 2005). Therefore, high Cu might be a general condition in this fjord, and indigenous plankton might have developed physiological mechanisms to deal with high Cu, such as the production of organic ligands to prevent uptake (Vraspir and Butler, 2009), or of heavy-metal-binding *peptides (phytochelatins) to lower Cu toxicity inside the cell (Ahner and Morel, 1995; Ahner et al., 1995; Knauer et al., 1998). Since we measured high particulate Cu, and Cu:P in our experiment, E. huxleyi might have been relying mainly on phytochelatins to buffer high intracellular Cu (Ahner et al., 2002).*

18) Line 344: "The Cd:P were significantly lower than those found in phytoplankton and E. huxleyi.". Reader can not understand clearly which data they compared. Is this sentence mean that "The Cd:P were significantly lower than those found in individual phytoplankton and E. huxleyi which was reported by previous studies (Ho, 2006, Ho et al., 2003)"?. If so, they should show the comparable data from previous study.

We have clarified this in section 4.3 Particulate metals with a strong biogenic component: their P-normalized ratios, Lns 364-372 :" *The Cd:P ratios (average 0.024 ± 0.01 mmol Cd:mol P, Figure 4 and 6) were significantly lower than those in phytoplankton and E. huxleyi (0.36 mmol Cd:mol P, Figure 4 and 6). This was surprising, because Cd quotas are normally higher in coccolithophores than in diatoms and chlorophytes (Sunda and Huntsman, 2000; Ho et al., 2003). High Cd quotas in coccolithophores have been suggested to result from accidental uptake through Ca transporters and channels (Ho et al., 2009). The low Cd quotas here may be explained by the antagonistic interaction between Mn and Cd or Zn and Cd under high Mn and Zn, respectively (Sunda and Huntsman, 1998, 2000; Cullen and Sherrell, 2005). Since high Zn:P ratios were common in this study (34.02 ± 18.05 mmol Zn:mol P, Figure 4 and 6), we hypothesize that high Zn levels antagonistically interacted with Cd, resulting in low Cd:P ratios in the particles"*

19)Line 377: "The decrease in particulate Fe might have been due to enhanced solubility of Fe- oxides at low pH.". The author should show scientific basis. They have to show relation between pFe and PH in each treatment.

It is now shown in Figure 1 and Figure S2 and discussed in 4.4 The effects of increased CO_2 and the DFB addition on particulate metal concentrations and P-normalized ratios, Lns 373-395:

"Iron enrichment is common in coastal waters, due to sediment resuspension, rivers input, aeolian deposition and mixing or upwelling of deep water. Indeed, Fe was the essential metal with the highest particulate concentrations in our study (Figure 3, Table 3). Furthermore, in this study particulate Fe was characterized by a strong lithogenic component, and was not correlated with phytoplankton biomass. Iron was also unique, in that it was the only trace element whose particulate concentration was significantly and uniquely affected by CO_2, regardless of the presence or absence of DFB (no interaction between CO_2 and DFB, Table 1). Furthermore, particulate Fe concentrations (nM) decreased steadily between days 12 and 21, with the exception of the control treatment (LC-DFB; Figure 3, Table 2S). This suggests that the increase in CO_2 and/or the DFB addition reduce the concentration of pFe, despite the phytoplankton bloom. Such a decrease in pFe (range 2.3-fold in LC-DFB, vs. 13.7-fold in HC+DFB; Table S2) might be mediated by the dissolution of particulate Fe by low pH or by the presence of strong organic chelators as observed in this very experiment (Segovia et al. 2017 and references therein). where dFe notably increased in treatments with high CO_2 and/or the addition of DFB (Figure 1). Furthermore, the dissolution of particulate Fe in the treatments with high

CO_2 and/or the addition of DFB was evident in the Fe partitioning coefficients—the molar ratio between particulate and dissolved concentrations. On day 21, the Fe partitioning coefficients varied by 22-fold between the highest for the control (LC-DFB: 1.039) and lowest for the HC+DFB treatments (HC+DFB: 0.047; Figure S2). Thus, either the DFB addition or high CO_2 promoted the dissolution of pFe. However, at the end of the experiment, high dFe concentrations were only observed in the treatments with the DFB additions, suggesting that the presence of strong organic Fe chelators, such as DFB, mediated the maintenance of high dissolved Fe concentrations, as previously observed (Segovia et al. 2017). Thus, in our future oceans, high CO_2 (low pH) will increase dissolved Fe concentrations in regions rich in particulate Fe, and in strong organic Fe chelators."

20)Line 378: "the concentration of the elements P, Co, Zn, Mn and Mo were influenced by CO2 and Fe levels". Which data indicate those results? Authors should present with their dataset. Line 380-381: "where the addition of DFB resulted in higher dissolved Fe, and optimal pH enhanced E. huxleyi growth.". Authors should present this relation, between dissolved Fe, pH and E. huxleyi growth, with figures which are constructed by their dataset.

This is now solved by inclusion of Fig 1, Fig 2 , by changing Tables to Figs 3 and 4 and answered in points 3,6,9 and 19.

21) conclusion remarks Please consider for my "general comment". It is necessary to describe more specifically what was understood in each argument (claim) a)-d).Authors should present what are difference/similarity of their data among four mesocosms treatment more clearly, and what they can find from the difference/similarity? How they induced the conclusion of this study form the difference/similarity? This aspect is not clear overall in this manuscript.

We believe that this requirement is now met attending to the deep-structural changes we have done in the ms.

22) Others Authors used "pFe", "particulate Fe", "particulate iron", "dFe", and "dissolved Fe" in the text. They should use same words through the manuscript.

Changed accordingly

Anonymous Referee #2

I found it very hard to follow the description of the data in the Results, as most of them have been presented in tables, which is especially not good for presenting the time dependent changes in for example trace metal concentrations. I also found that the Discussion for the most part was on trace metal chemistry and physiology in general, but not specifically relative to the key objective of the study, i.e., the effects of CO2 and Fe availability on particulate trace metal dynamics.

See point 1, 21 and others to Referee #1

Specific comments. Line 91. “(Hutchings, 2011)”, which was not included in the References.

Now included

Line 108. 10 uM nitrate: 0.3 uM PO4 = 33:1 - was there a particular reason to use such a P limited nutrient condition? How may this affect the observed particulate trace metal concentration?

Yes, we used this specific ratio because we aimed at a Emiliania huxleyi bloom. This has been clarified as follows, Lns 117-119:

At the beginning of the experiment, nitrate (10 μM final concentration) and phosphate (0.3 μM final concentration) were added to induce a bloom of the coccolithophore Emiliania huxleyi, as recommended by Egge & Heimdal (1994).

Results: I would strongly suggest that the data should be presented as figures instead of tables. In addition, albeit statistical analyses were conducted and presented together in Table 5, I would suggest they should also be presented in each individual table (or figure, if the authors decide to follow my suggestion above in revising the manuscript).

Done

Lines 170-181. “days 1-10, phase I”, “day 7” and “After day 10” were mentioned when describing the data, but none of them can be found in Table 2. Line 221. “(Figure 5)” should be Table 5.

This has now changed with the new Ms organisation.

Discussion: Again the Discussion mostly did not center around the influence of acidification and/or Fe availability on trace metal dynamics, except for the last, very short section 4.4. I thus encourage the authors to considerably revise the Discussion, focusing on how the chemistry and utilization of trace metals were affected by changes in CO2/pH and Fe levels and how these may be related to the proliferation of Ehux in the mesocosm.

This has now changed with the new Ms organisation. See comment to Referee 1.

We thank the reviewers for their comments and their time, and hope that our responses are satisfactory

Yours sincerely,

Maria Segovia & Maite Maldonado

Particulate trace metal dynamics in response to increased CO_2 and iron availability in a coastal mesocosm experiment

M. Rosario Lorenzo[1], María Segovia[1], Jay T. Cullen[2], and María T. Maldonado[3]

[1]Department of Ecology, Faculty of Sciences, University of Málaga, Bulevar Louis Pasteur s/n, 29071-Málaga, Spain
[2]School of Earth and Ocean Sciences, University of Victoria, 3800 Finnerty Road, Bob Wright Centre A405, Victoria
BC V8P 5C2
Canada
[3]Department of Earth, Ocean and Atmospheric Sciences, University of British Columbia, 2207 Main Mall, Vancouver
BC V6T 1Z4, Canada

Correspondence to: María Segovia (segovia@uma.es) and María T. Maldonado (mmaldonado@eoas.ubc.ca)

Abstract. Rising concentrations of atmospheric carbon dioxide are causing ocean acidification and will influence marine processes and trace metal biogeochemistry. In June 2012, in Raunefjord (Bergen, Norway) we performed a mesocosm experiment, comprised of a fully factorial design of ambient and elevated pCO_2 and/or an addition of the siderophore desferrioxamine B (DFB). In addition, the macronutrient concentrations were manipulated to enhance a bloom of the coccolithophore *Emiliania huxleyi*. We report here the changes in particulate trace metal (pMe) concentrations during this experiment. Our results show that particulate Ti and Fe were dominated by lithogenic material while particulate Cu, Co, Mn, Zn, Mo and Cd had a strong biogenic component. Furthermore, significant correlations were found between particulate concentrations (mol L^{-1}) of Cu, Co, Zn, Cd, Mn, Mo, and P in seawater and phytoplankton biomass (µgC L^{-1}), supporting a significant influence of the bloom in the distribution of these particulate elements. The concentrations of these biogenic metals (mol L^{-1}) in the *E. huxleyi* bloom were ranked as: Zn > Cu ≈ Mn > Mo > Co > Cd. Changes in CO_2 and/or DFB affected total particulate concentrations (mol L^{-1}) and biogenic metal ratios (Me:P) for some metals. Variations in CO_2 had the most clear, and significant effect on particulate Fe concentrations (mol L^{-1}), decreasing its concentration under high CO_2. Similarly, high CO_2 decreased the Co, Zn and Mn: P ratios, while increased the Cu: P ratios. In contrast, the addition of DFB had no significant effect on any of the biogenic metal ratios, whilst high concentrations of dissolved Fe will only be maintained by the presence of strong organic ligands. Future predicted high CO_2 levels are expected to change the relative concentrations of particulate and dissolved metals, due to the differential effects of high CO_2 on trace metal solubility, speciation, adsorption and toxicity, as well as on the growth of different phytoplankton taxa, and their elemental trace metal composition. These processes will also be mediated by the presence of strong organic ligands in areas where particulate Fe inputs are important, since the effectiveness of some natural chelators such as siderophores, in dissolving Fe from oxyhydroxides and/or by enhancing the photoinduced redox cycle of Fe, will be increased. This study demonstrates the utility and robustness of combining trace metal analyses of particles in a controlled mesocosm experiment with manipulations of CO2 and Fe concentrations using natural assemblages of marine phytoplankton in order to understand future ocean dynamics.

Key words: Global change, iron, CO_2, particulate trace metals, dissolved trace metals, mesocosms, *Emiliania huxleyi,* phytoplankton

Con formato: Inglés (británico)

Con formato: Inglés (británico)

Con formato: Inglés (británico)

Con formato: Inglés (británico)

Con formato: Inglés (británico)

Eliminado: and

Con formato: Inglés (británico)

Con formato: Inglés (británico)

Código de campo cambiado

Con formato: Color de fuente: Automático

Eliminado: eos

Con formato: Color de fuente: Automático

Con formato: Color de fuente: Automático, Inglés (británico)

Con formato: Color de fuente: Automático

Con formato: Inglés (británico)

Con formato: Punto de tabulación: 11.75 cm, Izquierda

Movido (inserción)[1]

Con formato: Inglés (británico)

Eliminado: The importance of the combined impacts of elevated CO_2 and changes in trace metal availability on marine plankton remain largely unknown. A mesocosm experiment was performed to study changes in particulate trace metal concentrations during a bloom dominated by the coccolithophore *Emiliania huxleyi*. We employed a full-factorial experimental design, comprising all combinations of ambient and elevated pCO_2 and dissolved iron (dFe). Particulate metal concentrations (Fe, Cu, Zn, Co, Mn, Cd, Mo, Ti and Pb) were determined by high-resolution inductively coupled plasma mass spectrometry (HR-ICPMS). We examined biogenic and lithogenic sources of particulate metals, and their evolution during the experiment. Biogenic metal concentrations were estimated from bulk particle measurements by comparing phosphorus (P)-normalised quotas with published ratios, as well as concentrations of particulate trace metals in the presence and absence of an oxalate-EDTA wash. Our results demonstrate that particulate Ti and Fe concentrations were dominated by lithogenic material in the fjord. In contrast, particulate Cu, Co, Mn, Zn, Mo and Cd concentrations correlated with P concentrations and phytoplankton biomass, indicative of their strong biogenic character. Furthermore, ocean acidification changed the relative concentrations of particulate metals; a result mainly driven by the effects of ocean acidification on the growth of different phytoplankton phyla.

Con formato: Inglés (británico)

Con formato: Inglés (británico), Sin Superíndice / Subíndice

Con formato: Inglés (británico)

Eliminado: .

Con formato: Fuente: Sin Negrita, Inglés (británico)

Con formato: Inglés (británico)

1. Introduction

Marine phytoplankton contribute half of the world's total primary productivity, sustaining marine food webs and driving the biogeochemical cycles of carbon and nutrients (Field et al., 1998). Annually, phytoplankton incorporate approximately 45 to 50 billion metric tons of inorganic carbon (Field et al., 1998), removing a quarter of the CO_2 emitted to the atmosphere by anthropogenic activities (Canadell et al., 2007). Yet, the atmospheric CO_2 concentration has increased by 40 % since pre-industrial times as a result of anthropogenic CO_2 emissions, producing rapid changes in the global climate system (Stocker et al., 2013). The dissolution of anthropogenic CO_2 in seawater, causes shifts in the carbonate chemical speciation, and leads to ocean acidification (OA). Marine ecosystems are sensitive to changes in pH because pH strongly affects chemical and physiological reactions (Hoffman et al., 2012). Increased CO_2 in seawater may enhance or diminish phytoplankton productivity (Mackey et al., 2015), decrease the $CaCO_3$ production in most planktonic calcifiers (Riebesell and Tortell 2011), and/or inhibit organic nitrogen and phosphorus acquisition (Hutchins et al., 2009). Thus, the biogeochemical cycling of nutrients is predicted to be highly affected by OA (Hutchins et al. 2009), as well as the distribution and speciation of trace metals in the ocean (Millero et al., 2009).

Trace metals, including Fe, Zn, Mn, Cu, Co and Mo, are essential for biological functions (e.g. photosynthesis, respiration and macronutrient assimilation), and Cd can supplement these functions. Trace metals availability can influence phytoplankton growth and community structure (Morel and Price, 2003). In turn, plankton control the distribution, chemical speciation, and cycling of trace metals in the sea (Sunda, 2012), by, for example, releasing organic compounds that dominate the coordination chemistry of metals, internalizing trace elements into the cells, and reducing and/or oxidizing metals at the cell surface. The chemistry of redox speciation of active trace metals is highly dependent on pH. For instance, Fe occurs in two main redox states in the environment: oxidized ferric Fe (Fe (III)), which is poorly soluble at circumneutral pH; and reduced ferrous Fe (Fe (II)), which is easily soluble and therefore more bioavailable. Fe speciation and bio- availability are dynamically controlled by the prevalent changing redox conditions. Also, as the ocean becomes more acidic, reduction of Cu (II) will increase, as the ionic form of Cu (II) is reduced to Cu (I) (Millero et al., 2009). The effect of higher concentrations of Cu (I) in surface waters on biological systems is not well known. Therefore, while the effects of OA on inorganic metal speciation will be more pronounced for metals that form strong complexes with carbonates (e.g. copper) or hydroxides (e.g. iron and aluminium), those that form stable complexes with chlorides (e.g. cadmium) will not be greatly affected. pH mediated changes in concentrations and/or speciation could possibly enhance trace metals limitation and/or toxicity to marine plankton (Millero et al., 2009).

Iron is crucial for phytoplankton growth because of its involvement in many essential physiological processes, such as photosynthesis, respiration, and nitrate assimilation (Behrenfeld and Milligan, 2013). The decrease in seawater pH in response to OA may increase Fe solubility (Millero et al., 2009), but it may also result in unchanged or lower Fe bioavailability, depending of the nature of the strong organic Fe ligands (Shi et al., 2010). Consequently, changes in iron bioavailability due to ocean acidification can affect positively or negatively ocean productivity and CO_2 drawdown. Copper is an essential micronutrient but may be toxic at high concentrations (Semeniuk et al., 2016). An increase in free cupric ion concentrations in coastal areas due to ocean acidification (Millero et al., 2009) could result in negative effects on phytoplankton. From the open-ocean to coastal areas, the concentration of metals differ, as well as the trace metal requirements of phytoplankton (Sunda and Huntsman, 1995a), and their tolerance to metal toxicity. Accordingly, changes in pH may promote an increase in Cu toxicity in coastal phytoplankton, or enhance Fe limitation in the open ocean. Given that trace metals are essential for phytoplankton productivity, and that are actively

Con formato: Inglés (británico)

Eliminado: phytoplankton

Con formato: Inglés (británico)

Eliminado:). In addition, variations in pH will affect

Con formato: Inglés (británico)

Con formato: Inglés (británico)

Eliminado: Thus, trace

Con formato: Inglés (británico)

Eliminado: elements

Con formato: Inglés (británico)

Con formato: Inglés (británico)

Eliminado: Thus,

Con formato: Inglés (británico)

Con formato: Inglés (británico)

Eliminado: metal

Con formato: Inglés (británico)

Con formato: Inglés (británico)

Con formato: Normal, Derecha: 0.04 cm

Con formato: Inglés (británico)

internalized during growth, it is important to study the impacts of ocean acidification in the trace metal content of ecologically significant plankton species.

In a rapidly changing global environment, generated by anthropogenic CO_2 emissions, it is critical to gain adequate understanding about ecosystem responses. Due to the complex interactions in aquatic ecosystems such predictions have so far not been possible to do based upon observational data and modelling alone. However, direct empirical studies on natural communities offer a robust tool to analyse interactive effects of multiple stressors. Specifically, mesocosm experiments allow perturbation studies with a high degree of realism compared to other experimental systems (Riebesell et al., 2010, Stewart et al., 2013, Riebesell and Gatusso, 2015).

In the present work a bloom of the coccolithophorid *Emiliania huxleyi* was induced in a mesocosm experiment to examine the interactive effects of increased CO_2 and/or dissolved iron on its growth and physiology (Segovia et al., 2017, Segovia et al., 2018, Lorenzo et al., 2018). *Emiliania huxleyi* is the most cosmopolitan and abundant coccolithophore in the modern ocean (Paasche, 2002). Coccolithophores play a key role in the global carbon cycle because they produce photosynthetically organic carbon, as well as particulate inorganic carbon through calcification. These two processes foster the sinking of particulate organic carbon to the deep ocean carbon export (Hutchings, 2011) and impact organic carbon burial in marine sediments (Archer, 1991, Archer and Maier-Reimer., 1994). However, ocean acidification will disproportionally affect the abundance of coccolithophores, as well as their rates of calcification and organic carbon fixation (Zondervan et al., 2007). The aim of the present study was to characterize the changes in particulate trace metal concentrations during the bloom of *E. huxleyi* given realistic changes in CO_2 and Fe bioavailability.

2. Materials and methods

2.1 Experimental set-up

The experimental work was carried out in June 2012 in the Raunefjord, off Bergen, Norway as described in detail by Segovia et al., (2017). Twelve mesocosms (11 m^3 each) were set-up in a fully factorial design with all combinations of ambient and elevated pCO_2 and dFe in three independent replicate mesocosms. The mesocosms were covered by lids (both transparent to PAR and UVR) and filled with fjord water from 8 m depth. We achieved two CO_2 levels corresponding to present (390 ppm, LC) and those predicted for 2100 (900 ppm, HC) by adding different quantities of pure CO_2 gas (Shculz et al., 2009). The specific CO_2 concentration and the CO_2 inlet flows in the mesocosms were measured by non-dispersive infrared analysis by using a Li-Cor (LI-820) CO_2 gas analyser (Li-COR, Nebraska, USA). CO_2 concentrations in the mesocosms were calculated from pH and total alkalinity measurements using the CO_2 SYS software (Robbins et al,. 2010). At the beginning of the experiment, nitrate (10 µM final concentration) and phosphate (0.3 µM final concentration) were added to induce a bloom of the coccolithophore *Emiliania huxleyi*, as recommended by Egge & Heimdal (1994). To induce changes in Fe availability, and analyse its effects on the plankton community, 70 nM (final concentration) of the siderophore desferrioxamine B (DFB) (+DFB and −DFB treatments) (Figure 1b) was added to half of the mesocosms on Day 7, when the community was already acclimated to high CO_2. The initial dFe concentration before DFB addition was about 4.5 nM. Even though DFB is a strong Fe-binding organic lig- and often used to induce iron limitation in phyto- plankton (Wells 1999), DFB additions may also in- crease the dissolved Fe pool in environments with high concentrations of colloidal and/or particulate Fe, such as fjords (Kuma et al. 1996, Öztürk et al. 2002). By day 17, dissolved iron concentrations were significantly higher (by ~3-fold) in the high CO_2 and DFB treatments than in the control (Segovia et al. 2017). These results support an increase in the solubility of Fe in seawater by either lowering its pH (Millero 1998; Millero et al. 2009) and/or the addition of DFB (Chen et al. 2004). The

Eliminado: OA
Con formato: Inglés (británico)
Eliminado: 2
Con formato: Inglés (británico)
Con formato: Ninguno, Derecha: 0 cm, Espacio Después: 12 pto, Agregar espacio entre párrafos del mismo estilo, No ajustar espacio entre texto latino y asiático, No ajustar espacio entre texto asiático y números, Punto de tabulación: No en 11.75 cm
Eliminado: Shulz
Con formato: Inglés (británico)
Eliminado:) and CO_2 AirSense-310 sensors (Digital Control Systems, Inc, USA).
Con formato: Inglés (británico)
Con formato: Inglés (británico)
Con formato: Inglés (británico)
Con formato: Inglés (británico)
Eliminado: concentrations
Con formato: Inglés (británico)
Eliminado: manipulated
Con formato: Inglés (británico)
Eliminado: . Iron was also manipulated
Con formato: Inglés (británico)
Eliminado: addition of
Con formato: Color de fuente: Color personalizado(RGB(21;21;24))
Con formato: Color de fuente: Color personalizado(RGB(21;21;24))
Con formato: Color de fuente: Color personalizado(RGB(21;21;24))
Eliminado: day
Con formato: Color de fuente: Color personalizado(RGB(21;21;24))
Eliminado: promoting two different
Con formato: Color de fuente: Color personalizado(RGB(21;21;24))
Eliminado: conditions (+DFB, high
Con formato: Color de fuente: Color personalizado(RGB(21;21;24))
Eliminado: iron; and –DFB, ambient dissolved iron).
Eliminado: ~3-fold
Con formato: Color de fuente: Color personalizado(RGB(21;21;24))
Con formato: Inglés (británico)
Con formato: Inglés (británico)
Con formato: Inglés (británico)
Con formato: Inglés (británico)
Eliminado: .
Con formato: Inglés (británico)

multifactorial experimental design consisted of triplicate mesocosms per treatment and the combinations of high and ambient pCO$_2$ and dFe levels, resulted in a total of 12 mesocosms: LC-DFB (control), LC+DFB, HC+DFB and HC-DFB. Water samples from each mesocosm were taken from 2 m depth by gentle vacuum pumping of 25 L volume into acid-washed carboys that were quickly transported to the onshore laboratory. The biological and chemical variables analysed were phytoplankton abundance and species composition, dissolved Fe and Cu concentrations (dFe, dCu), nutrient concentrations (nitrate, phosphate, silicic acid and ammonium) and particulate trace metal concentrations.

2.2 Dissolved copper (dCu)

Low density polyethylene (LDPE) bottles were cleaned with 1% alkaline soap solution for one week, then filled with 6 M trace metal grade HCl and submerged in a 2 M HCl bath for one month. For transport, they were filled with 1 M trace metal grade HCl (Fisher Chemicals) for one more month and kept double bagged. In between each acid treatment, the bottles were rinsed with Milli-Q water (Millipore; hereafter referred to as MQ). Before sampling, the bottles were rinsed three times with filtered seawater. Seawater was collected from each mesocosm, filtered through 0.2 µM AcroPak Supor membrane capsule filters into the trace metal clean LDPE bottles, and acidified with ultra-clean HCl (Seastar) in a Class 100 laminar flow hood. Total dissolved Cu concentrations were measured following Zamzow et al., (1998) using a flow injection analysis chemiluminescence detection system (CL-FIA, Waterville Analytical). Total dissolved Fe concentrations were measured as described in Segovia et al., (2017) for this very experiment.

2.3 Particulate metals (pMe)

2.3.1 Sampling

All equipment used during this study was rigorously acid-washed under trace metal clean conditions. Filters were precleaned with 10% trace metal hydrochloric acid (Fisher, trace metal grade) at 60°C overnight and were rinsed with Milli-Q (MQ) water. Seawater samples (1-3.5 L) were filtered gently onto 0.45 µm acid washed Supor ®-450 filters (within a trace metal clean Swinnex filter holder) on days 12, 17 and 21 of the experiment. Four technical replicates were taken from each mesocosm. Two filters were analysed without manipulation and the other two were individually washed with oxalate-EDTA reagent to remove extracellular Fe, as well as other metals (Tang and Morel, 2006). Immediately following filtration, the treated filters were soaked with 20 mL EDTA–oxalate solution, added to the headspace of the Swinnex holders, with an acid-washed polypropylene syringe. After 10 min, vacuum was applied to remove the oxalate solution and 10 mL of 0.2 µM filtered chelexed synthetic oceanic water (SOW) solution was passed through the filter to rinse off any remaining oxalate solution. Replicate filters that were not treated with oxalate solution were transferred directly to centrifuge tubes for storage. The filters with particles were frozen in acid-washed 2 mL PP tubes and then, dried and stored until analysis.

2.3.2 Analytical methods

Filters were digested in 7-mL acid-washed Teflon (Teflon, Rochester, NY, USA) vials. Teflon vials were also precleaned using 10% trace metal hydrochloric acid (Fisher, trace metal grade) during two days and then, with nitric acid (Fisher, trace metal grade) at 70 ºC during three days. In between each acid treatment, the bottles were rinsed with MQ. Samples were digested in 3 mL of HNO$_3$ and 0.5 mL of HF (Fisher, trace metal grade) with lids on for 1 h on a hot plate at 200 ºC. The lids were then removed to evaporate HF at 200ºC. After this, 1.5 mL of HNO$_3$ were added and the samples were heated with lids on overnight at 150 ºC. Finally, 2.25 mL of HClO$_4$ (Fisher, Optima grade) were added and the samples were heated for 4 h at 200 ºC. After complete digestion, the samples were dried on hot plates at 200ºC. The dried samples were dissolved in 1% nitric acid with 1 ppb in internal standard. The analysis was performed

Eliminado: resulting
Con formato: Inglés (británico)
Eliminado:
Con formato: Inglés (británico)
Con formato: Fuente: Times, 12 pto, Sin Cursiva
Con formato: Inglés (británico)
Eliminado: 3
Con formato: Inglés (británico)
Con formato: Inglés (británico)
Eliminado: 4
Con formato: Inglés (británico)
Con formato: Inglés (británico)
Eliminado: 4
Con formato: Inglés (británico)
Eliminado:
Con formato: Inglés (británico)
Con formato: Inglés (británico)
Con formato: Inglés (británico)
Con formato: Inglés (británico)
Con formato: Inglés (británico)
Eliminado: in an
Con formato: Inglés (británico)
Eliminado: by adding 20 mL of oxalate solution
Con formato: Inglés (británico)
Con formato: Inglés (británico)
Eliminado: 4
Con formato: Inglés (británico)

using a high-resolution inductively coupled plasma-mass spectrometer (ICP-MS, Element XR, Thermo Scientific) and the described instrumental settings (Table S1). Filter blanks were collected and subjected to the same storage, digestion, dilution, and analysis processes, and these blank values were subtracted from sample measurements. Particulate samples for ICPMS analysis were processed in a trace metal-clean laboratory under a trace metal-clean laminar flow fume hood.

2.3.3 The effect of oxalate-EDTA wash on particulate trace metal concentrations

To better estimate the biogenic fraction of the particulate metals, the filters were washed with an oxalate-EDTA solution, which removes extracellular metals and oxyhydroxides (Tovar-Sanchez et al., 2003; Tang and Morel, 2006). In our study, the oxalate wash significantly decreased the concentration of all particulate metals, with the exception of Al and Ti (Tables S2 and S3), as observed by Rauschenberg and Twining (2015). The quantity of metal remaining after the oxalate wash (i.e. biogenic fraction) varied among elements (Tables S2 and S3). In general, the concentrations of Fe and Co in the particles were decreased the least by the oxalate wash by ~ 25%, while Mo and Pb concentrations were decreased the most by ~70%. The concentrations of particulate Cu, Zn, Cd and Mn were reduced by 50% by the oxalate wash. As shown previously (Sanudo-Wilhelmy et al. 2004), the oxalate reagent also removed extracellular P (by ~20%, Table S2 & S3). Compared to Rauschenberg and Twining (2015), the estimates of the biogenic fraction, after the oxalate wash, were in agreement for Co, Cu and P, and lower for Fe, Mn, Zn and Cd concentrations.

However, the efficacy of the oxalate wash to dissolve Fe, and other metals, from lithogenic particles is not well constrained (Frew et al. 2006, Rauschenberg and Twining, 2015, King et al., 2012). Therefore, the results obtained after the oxalate-EDTA wash should be interpreted with caution because we do not know whether the removed metal fraction is a) only lithogenic; b) mainly lithogenic but some biogenic fraction is also removed, or c) whether metals absorbed onto particles are equally labile to the wash on biogenic and lithogenic particles. Given that many of the trends we observed were identical for the oxalate-EDTA washed and non-washed particles [i.e. higher Me concentrations in the LC+DFB treatments (Table S2 & S3) and positive correlations between phytoplankton biomass and Me concentrations (Lorenzo-Garrido 2016)], below we present and discuss only the non-oxalate wash results.

2.4 Statistical analyses

Data were checked for normality (by Shapiro-Wilks' test), homoscedasticity (by Levene's test) and sphericity (by Mauchly's test). All data met the requirements to perform parametric tests. Statistical significance of treatment effects was carried out using Split-Plot ANOVA followed by post-hoc Sidak and Bonferroni tests (considering $P < 0.05$ as significant). All analyses were performed using the General Linear Model (GLM) procedure. The correlation between variables was analysed by Pearson's product-moment multiple comparisons (considering $P < 0.05$ as significant). Statistical analyses were carried out using SPSS v22 (IBM statistics) and Sigmaplot 12 (Systat Software, Chicago, USA).

Eliminado: 1
Con formato ... [1]
Con formato ... [2]
Eliminado: 5
Movido hacia abajo[2]: **Statistical analyses**¶
Eliminado: **changed during the Emiliania huxleyi bloom**
Con formato ... [4]
Movido hacia abajo[3]: **3. Results** ¶
Con formato ... [5]
Eliminado: All the biological and chemical variables ... [6]
Eliminado: day 8 (Segovia et al., 2017). During the first ... [8]
Movido hacia abajo[4]: This bloom was not observed
Con formato ... [3]
Con formato ... [7]
Con formato ... [11]
Con formato ... [9]
Eliminado: the exception was the HC-DFB treatment ... [10]
Con formato ... [12]
Eliminado: The particulate trace-metal concentrations ... [13]
Con formato ... [14]
Eliminado: Tang and Morel., 2006). The only two elements ... [15]
Con formato ... [16]
Eliminado: ¶ ... [17]
Movido hacia abajo[5]: ¶
Con formato ... [18]
Eliminado: We investigated changes in particulate trace ... [19]
Movido hacia arriba[1]: *huxleyi* bloom were ranked as:
Con formato ... [20]
Eliminado: Changes in CO_2 and/or Fe levels affected total ... [21]
Con formato ... [22]
Eliminado: Table 4
Con formato ... [23]
Eliminado: (
Con formato ... [24]
Eliminado: %),
Con formato ... [25]
Eliminado: (
Con formato ... [26]
Eliminado: %).
Con formato ... [27]
Eliminado: %).
Con formato ... [28]
Eliminado: The
Con formato ... [29]
Eliminado: However, some
Con formato ... [30]
Eliminado: [eg.
Con formato ... [31]
Eliminado: 4); or
Con formato ... [32]
Eliminado: Table 6)] were identical for the oxalate-EDTA ... [33]
Con formato ... [34]
Eliminado: focus our discussion on
Con formato ... [35]
Con formato ... [36]
Con formato ... [37]
Movido (inserción)[2]

3. Results

3.1 Biological and chemical characteristics during the bloom

Plankton community dynamics and their response to the applied treatments in the mesocosms are described in detail by Segovia *et al.* (2017). Briefly, at the beginning of the experiment (days 1-10) a bloom of large chain-forming diatoms was observed, which declined by day 7 (Figure 2). This diatom bloom was associated with a sharp decrease in nitrate and silicic acid concentrations (Figure S1-supplemental material). Picoeukaryotes, dominated the phytoplankton community on day 8 (Figure 2). During the first 10 days of the experiment, there were no significant differences in the chemical variables measured between the treatments (Figures 1 and S1). On day 7, half of the mesocosms were amended by adding DFB (+DFB treatments). Between day 7 and 17, an increase in dFe was observed in all treatments, except in the control (Figure 1). This increase in dFe was sustained for the entire experiment in the DFB treatments (Figure 1). Dissolved Cu concentrations were not affected by the different treatments (Figure 1). After day 10, a massive bloom of the coccolithophore *Emiliania huxleyi* developed under LC +DFB condition (Figure 2), out-competing the rest of the plankton groups (Figure 2). This bloom was not observed either in the control treatment (LC-DFB) or in the HC treatments, although *E. huxleyi* was still the most abundant species in all treatments; with the exception of the HC-DFB treatment (Figure 2).

3.2 Particulate metal concentrations during the mesocosm experiment

The pMe concentrations (nM, mean of all treatments and dates) during the experiment were highest for Al, Fe and Zn, and lowest for Cd, following this trend: Al ≈ Fe ≈ Zn > Ti > Cu ≈ Mn > Mo ≈ Pb > Co > Cd (Figure 3, Table S2). Significant changes over time were observed for all particulate trace metal concentrations (Fe, Cu, Co, Zn, Cd, Mn, Mo and Pb), except for Ti and Al (Figure 3, Table 1). The only metal that showed a significant time-dependent decrease in its particulate concentration was Fe (Figure 3, Table 1). In general, the treatments with the highest particulate metals concentrations also exhibited the highest particulate P, except for Al, Ti, Fe, and Pb (Figure 3, Table S2). On days 12 and 17, the highest particulate metals concentrations were observed in the LC+DFB treatment, while on day 21, they were observed in both LC treatments (Figure 3, Table S2).

3.3 The effects of increased CO_2 and the DFB addition on particulate metal concentrations

Increased CO_2 and the DFB addition did not significantly affect the concentrations of particulate Al, Ti, Cu, and Pb (Tables 1and S2). Similarly, the addition of DFB did not directly influence particulate concentrations of Fe, but high CO_2 had a significant negative impact on particulate Fe (Tables 1 and S2, Figure 3). Particulate Cd concentrations were also inversely affected by CO_2, but only in the presence of DFB (CO_2; and CO_2 x DFB effect, Tables 1 and S2, Figure 3). All other elements (P, Co, Zn, Mn and Mo) exhibited significant effects by CO_2 and by DFB, but there was also a significant interaction between these two factors (Table 1, S2). This indicates that, for example, particulate Mn, Zn, Mo, Co, and P concentrations were significantly decreased by high CO_2, but only in the +DFB treatments (Figure 3, Table 1, S2,). Similarly, the addition of DFB significantly increased pZn and pMn, but only at ambient CO_2 levels (Figure 3, Table 1, S2).

3.4 Phosphorous-normalized metal ratios in particles collected from the mesocosms and the effects of increased CO_2 and the DFB addition on these ratios

The P-normalized metal ratios (Figure 4 and means in Table 2) were highest for Al and Fe (mean: 70 ± 38 mmol Al: mol P, and 39 ± 34 mmol Fe: mol P), and lowest for Cd and Co (mean 0.02 ± 0.01 mmol Cd: mol P, and 0.07 ± 0.02 mmol Co: mol P). Iron:P and Ti:P were not significantly affected by increased CO_2 and/or the DFB addition, but showed a significant decrease over time (Table 3). The P-normalized Cu, Co and Zn ratios changed significantly over

Movido (inserción)[3]

Con formato: Inglés (británico)

Con formato: Fuente: 12 pto, Inglés (británico)

Movido (inserción)[4]

Con formato: Inglés (británico)

Con formato: Inglés (británico)

Con formato: Fuente: Sin Negrita, Inglés (británico) time (Table 3). Increased CO_2 significantly decreased Co, Zn and Mn:P ratios, while it increased Cu:P ratios (Figure 4, Table 3). DFB did not affect the Me:P ratios of any of these bioactive elements (Table 3).

4. Discussion

4.1 The effects of CO_2 and dFe in the plankton community

In this experiment we investigated changes in particulate trace metal concentrations, in response to increased CO_2 and/or an addition of the siderophore DFB in a coastal mesocosm experiment. For a better understanding of the processes affecting these stressors, we briefly summarise the mesocosm experiment results originating from Segovia et al. (2017). High CO_2, as well as the DFB addition increased dFe concentration. The higher dFe concentrations were sustained in the DFB treatments. A bloom of the coccolithophore *Emiliania huxleyi* was observed in the ambient CO_2 treatments, and was especially massive in the one with the addition of DFB (LC+DFB). On the contrary, the biomass of *E. huxleyi* was negatively affected by increased CO_2. However, increased dFe partially mitigated the negative effect of elevated CO_2, indicating that the coccolithophore was able to acclimate better to ocean acidification when Fe availability was high. High dFe also had a positive effect on the cyanobacterium *Synechococcus sp*, while the rest of the plankton food web did not response to the treatments (Segovia et al. 2017).

4.2 Particulate Fe and Ti are associated with lithogenic sources, while particulate Co, Cu, Zn, Cd, Mo and Mn are associated with biogenic sources

The particulate trace metal concentrations (nM, mean of all treatments and dates) during the experiment were highest for Al, Fe and Zn, and lowest for Co and Cd, following this trend: Al ≈ Fe ≈ Zn > Ti > Cu ≈ Mn > Mo ≈ Pb > Co > Cd. Lithogenic particles are enriched in Al and low in P (average crustal Al and P content is 2.9 mmol Al and 0.034 mmol P g^{-1} dry weight, Taylor 1964), while biogenic particles are enriched in P and low in Al (average plankton Al and P content is 0.001 mmol Al and 0.26 mmol P g^{-1} dry weight, Bruland et al. 1991). Therefore, the distinct high abundance of Al and P in lithogenic and biogenic particles, respectively, can be used to evaluate the relative contribution of lithogenic and biogenic material in our particulate samples. In order to do this, first, it is important to establish that the vast majority of the measured particulate P is associated the biogenic fraction. In this study, the abiotic P was estimated using particulate Al concentrations (nM) and the P:Al ratio in crustal material, and was calculated to be negligible (< 1% of the total measured particulate P). In addition, a significant correlation ($p < 0.003$) was found between particulate P concentrations and phytoplankton biomass (Table 4). Therefore, we assume a constant trace metal composition in biogenic particles (assuming they are rich in phytoplankton) and lithogenic particles (assuming they are rich in crustal material). We then calculated the expected metal concentrations in the particulate samples assuming that all the P measured in the particles is associated with a biogenic fraction, and that all Al in the particles is associated with the lithogenic fraction. Thus, for a given trace metal, its expected particulate trace metal concentration in seawater (mol L^{-1}) can be calculated as the sum of the contribution from biogenic and lithogenic particles, so that:

$$[Me] = a\,[P] + b\,[Al]$$

where [Me] is the total concentration of the metal (mol L^{-1}) expected in the particulate sample; [P] is the P concentration measured in the particles (mol L^{-1}); [Al] is the Al concentration measured in the particles (nM L^{-1}); *a* is the average, well-known metal content in biogenic particles, normalized to P (i.e. mol Me: mol P in marine plankton) and *b* is the average, well known metal content in lithogenic particles, normalized to Al (mol Me: mol Al in the Earth crust). For example, on day 21 in the HC-DFB treatment, the concentrations of particulate Al and P were 8.22 and 134.8 nM, respectively (Table S2). Assuming a constant 0.0051 mol Fe: mol P in biogenic particles and 0.331 mol Fe:

Movido (inserción)[5]

Con formato: Inglés (británico)

Con formato: Fuente: Negrita, Inglés (británico)

Eliminado: **Cu, Co, Zn, Cd, Mn and Mo) were**

mol Al in lithogenic particles (Table 2), we calculated an expected particulate Fe concentration of 3.41 nM, where 80% was associated with lithogenic material and 20% with biogenic material. Similar calculations were made for the bioactive metals Mn, Co, Cu, Zn, Cd, and Mo (Table 5). Our calculations indicate that on average, particulate Fe was dominated by the lithogenic component (accounting for an average of 78% of the total expected particulate Fe), while for particulate Co, Cu, Zn, Cd, and Mo the biogenic fraction dominated (accounting for 94, 95, 99, 94 and 98%, respectively, of the total expected concentration; Table 5). Particulate concentrations of Mn were also dominated by the biogenic fraction (65% of the total), but the lithogenic fraction was also significant (35%). Moreover, the expected particulate Mn and Fe concentrations closely matched the particulate Mn and Fe concentration we measured (accounting for an average of ~ 71% of the measured Mn, and 115% of the measured Fe). For other metals (i.e. Cu, Mo and Zn), the expected particulate concentrations (nM) were lower than measured (23% of the measured pCu, and 8% of measured pZn; Table 5). This suggests that the particles were enriched in Cu, Mo, and Zn relative to what is expected based on natural marine plankton metal quotas (Bruland et al. 1991) and crustal ratios (Taylor 1964).

To further establish the lithogenic or biogenic source of the pMe in the particles, the particulate metal concentrations were normalized to the concentrations of particulate P and Al (Figure 4, and Table 2). These ratios were then compared with well-known molar ratios of metal to Al in the crust (Taylor 1964) and of metal to P ratios in marine plankton samples (Ho 2006) and cultures (Ho et al. 2003) (Table 2). The average Fe: Al (506 mmol Fe: mol Al) and Ti:Al ratios (119 mmol Ti: mol Al, Table 2) were relatively similar to crustal molar ratios (331 mmol Fe: mol Al and 39 mmol Ti: mol Al; Taylor 1964). Additional evidence for the significant lithogenic component in particulate Ti and Fe was gathered from Figure 5, where we plotted the molar ratios of the metals relative to P in the collected particles against the Al:P ratios measured in those same particles. The slope of these data [(Fe:P)/(Al:P) = mol Me: mol Al] is the ratio of Me:Al in the particles and can be compared to well-known Me:Al crustal ratio. Visually, if the data nicely fit the Me:Al line for crustal material, these metals are mainly associated with the lithogenic component, as evident for Fe and Ti (Figure 5). These combined results suggest that in our experiment, particulate Fe and Ti concentrations were enriched by lithogenic material. In support of this finding, we also found no significant correlation between particulate Fe and Ti concentrations (nM) and either the total plankton (phytoplankton and microzooplankton) or *E. huxleyi* biomass (μg C L^{-1}; Table 4).

In contrast, when the P-normalized metal ratios in the particles collected from the mesoscosms were plotted against the Al:P ratios in these particles, there were no correlations for the following metals Co, Cu, Zn, Cd, Mn and Mo (Figure 5), indicating that these particulate metals were not enriched in lithogenic material. Our measured metal: P ratios were comparable to plankton ratios in natural samples and in cultures (Table 5). The concentrations (mol L^{-1}) of these metals (i.e. Cu, Co, Zn, Cd, Mn, Mo), as well as P, also showed significant correlations with the biomass (μgC L^{-1}) of *E. huxleyi* and that of total plankton cells ($p < 0.05$, Table 4), supporting a significant influence of the phytoplankton in the distribution of these particulate elements.

4.3 Particulate metals with a strong biogenic component: their P-normalized ratios

The concentrations of particulate bioactive metals (mol L^{-1}), with a significant biogenic component (i.e. excluding Fe) in the studied *Emiliania huxleyi* bloom were ranked as: Zn > Cu ≈ Mn > Mo > Co > Cd (Figure 3, Table S3), similar to those reported in indigenous phytoplankton populations: Fe ≈ Zn > Cu ≈ Mn ≫ Co ≈ Cd, (Twining and Baines, 2013). The only treatment where E. *huxleyi* did not dominate the community was the HC-DFB; in this treatment the ranking of these biogenic particulate trace metals was the same as that of LC+DFB (with the massive *Emiliania huxleyi* bloom).

Con formato: Fuente: Sin Negrita, Inglés (británico)

Eliminado: **phytoplankton**

Con formato: Fuente: Sin Negrita, Inglés (británico)

Movido (inserción)[6]

Con formato: Inglés (británico)

Eliminado: Certain particulate elements (P, Cu, Co, Zn, Cd, Mn and Mo) were clearly biogenic. Three lines of evidence are presented in support of this. First, the total biomass of phytoplankton exhibited a significant positive correlation with particulate P (Table 6), suggesting that most particulate P was biogenic, as shown previously (Ho et al., 2007, Ho et al., 2009). The concentrations of Cu, Co, Zn, Cd, Mn and Mo also exhibited positive significant correlations with the biomass of total cells (phytoplankton and microzooplankton) or *E. huxleyi* (Table 6), indicating that these particulate metals were also associated with phytoplankton. Second, the Me:P ratios are not similar to crustal ratios. Third, the Me:P ratios we measured in the particles are similar to those of natural phytoplankton assemblages (Ho, 2006) and of *Emiliania huxleyi* cultures (Ho et al., 2003).¶
¶

Eliminado: biogenic metals

Con formato: Inglés (británico)

Con formato: Inglés (británico)

Con formato: Inglés (británico)

Eliminado: 3

Con formato: Inglés (británico)

Con formato: Inglés (británico)

Eliminado: ≈ Ni ≈ Cu

Con formato: Inglés (británico)

Eliminado: Particulate Zn concentrations were especially high in the LC+DFB treatment, where the highest *E.*

Movido hacia abajo[7]: *huxleyi* biomass was observed.

Con formato: Inglés (británico)

Eliminado: . In

Con formato: Inglés (británico)

Eliminado: ,

Con formato: Inglés (británico)

Eliminado: particulate trace metal

Con formato: Inglés (británico)

Con formato: Inglés (británico)

Eliminado: ,

Con formato: Inglés (británico)

but their concentrations were lower than those in LC+DFB. At the end of the experiment, the concentrations of these biogenic metals were, in general, comparable in both HC treatments, and lower than those in the LC treatments (Figure 3, Table S3). Therefore, high CO$_2$ had a tendency to decrease particulate metal concentrations, especially on day 21. Given the strong correlation between concentrations of these particulate bioactive metals and phytoplankton biomass, the lower particulate concentrations in high CO$_2$ were mainly due to low phytoplankton biomass.

Particulate Zn concentrations were especially high in the LC+DFB treatment (Figure 3), where the highest *E. huxleyi* biomass was observed. *Emiliania huxleyi* is well known for its high Zn cellular requirements (~ 1-10 for *E. huxleyi* vs. 1-4 mmol Zn: mol P for other phytoplankton; Sunda and Hunstman 1995, Sunda 2013). But, the Zn: P ratios in the LC+DFB treatment (range 45-69 mmol Zn: mol P; Figure 4, Table S2), as well as in all the other treatment (range 16-34 mmol Zn: mol P; Figure 4, Table S2) were significantly higher than these published ratios. This could be explained by, the adsorption of these metals to the outside of the cells, and/or anthropogenic inputs of Zn into the fjord. The Zn:P ratios in the samples washed with the oxalate-EDTA were still high (range 28-57 for LC+DFB and 16-33 mmol Zn: mol P in all other treatments, Table S3), thus adsorption might have not been significant. We hypothesize that anthropogenic aerosols which are rich in anthropogenic particulate metals, such as Zn and Cu (Perry et al. 1999; Narita et al. 1999), and have high percentage of Zn and Cu dissolution (ref.), might be the source of these high Zn concentrations and ratios in the particles.

Similarly, the Cu:P ratios in the collected particles were relatively elevated (1.4 ± 0.8 mmol Cu: mol P) compared to those of other phytoplankton, including *E. huxleyi* (Table 2). The dissolved (7.7±0.41 nM Cu, Figure 1) and particulate Cu concentrations (0.35±0.25 nM, Table S2) in our experiment were high, and similar to those previously measured in this fjord (Muller et al., 2005). Rain events (or wet deposition of anthropogenic aerosols) in this fjord result in high dissolved Cu and the active production of strong organic ligands by *Synechococcus*—to lower the free Cu concentrations (Muller et al., 2005). Therefore, high Cu might be a general condition in this fjord, and indigenous plankton might have developed physiological mechanisms to deal with high Cu, such as the production of organic ligands to prevent uptake (Vraspir and Butler, 2009), or of heavy-metal-binding peptides (phytochelatins) to lower Cu toxicity inside the cell (Ahner and Morel, 1995; Ahner et al., 1995; Knauer et al., 1998). Since we measured high particulate Cu, and Cu:P in our experiment, *E. huxleyi* might have been relying mainly on phytochelatins to buffer high intracellular Cu (Ahner et al., 2002).

The Cd:P ratios (average 0.024 ± 0.01 mmol Cd:mol P, Figure 4 and 6) were significantly lower than those in phytoplankton and *E. huxleyi* (0.36 mmol Cd:mol P, Figure 4 and 6). This was surprising, because Cd quotas are normally higher in coccolithophores than in diatoms and chlorophytes (Sunda and Huntsman, 2000; Ho et al., 2003). High Cd quotas in coccolithophores have been suggested to result from accidental uptake through Ca transporters and channels (Ho et al., 2009). The low Cd quotas here may be explained by the antagonistic interaction between Mn and Cd or Zn and Cd under high Mn and Zn, respectively (Sunda and Huntsman, 1998, 2000; Cullen and Sherrell, 2005). Since high Zn:P ratios were common in this study (34.02 ± 18.05 mmol Zn:mol P, Figure 4 and 6), we hypothesize that high Zn levels antagonistically interacted with Cd, resulting in low Cd:P ratios in the particles.

4.4 The effects of increased CO$_2$ and the DFB addition on particulate metal concentrations and P-normalized ratios

Iron enrichment is common in coastal waters, due to sediment resuspension, rivers input, aeolian deposition and mixing or upwelling of deep water. Indeed, Fe was the essential metal with the highest particulate concentrations in our study

Eliminado: higher
Con formato ... [38]
Eliminado: HC
Con formato ... [39]
Eliminado: on day 17
Con formato ... [40]
Eliminado: metal
Con formato ... [41]
Eliminado: in both HC treatments
Con formato ... [42]
Con formato ... [43]
Eliminado: and, similar effect of
Con formato ... [44]
Eliminado: factors (increased CO$_2$ and the addition of ... [45]
Con formato ... [46]
Movido (inserción)[7]
Con formato ... [47]
Eliminado: *Emiliania huxleyi* is well known for its high Zn ... [48]
Con formato ... [49]
Eliminado: ¶ ... [50]
Con formato ... [51]
Eliminado: Given that
Con formato ... [52]
Con formato ... [53]
Eliminado: but comparable to previous studies (Muller et ... [54]
Con formato ... [55]
Eliminado: or on other detoxification mechanisms
Con formato ... [56]
Eliminado: Indeed, in the genome of *E.huxleyi*, we found a ... [57]
Con formato ... [58]
Eliminado: Alternatively, Cu might be particularly ... [59]
Con formato ... [60]
Con formato ... [61]
Con formato ... [62]
Eliminado: found
Con formato ... [63]
Con formato ... [64]
Eliminado: .
Con formato ... [65]
Eliminado: The high Zn:P ratios in this study indicate high ... [66]
Con formato ... [67]
Movido hacia arriba[6]: ¶
Con formato ... [68]
Con formato ... [69]
Eliminado: **Fe in bulk particles was mainly lithogenic**¶
Con formato ... [70]
Eliminado: The
Con formato ... [71]

(Figure 3, Table 3). Furthermore, in this study particulate Fe was characterized by a strong lithogenic component, and was not correlated with phytoplankton biomass. Iron was also unique, in that it was the only trace element whose particulate concentration was significantly and uniquely affected by CO_2, regardless of the presence or absence of DFB (no interaction between CO_2 and DFB, Table 1). Furthermore, particulate Fe concentrations (nM) decreased steadily between days 12 and 21, with the exception of the control treatment (LC-DFB; Figure 3, Table 2S). This suggests that the increase in CO_2 and/or the DFB addition reduce the concentration of pFe, despite the phytoplankton bloom. Such a decrease in pFe (range 2.3-fold in LC-DFB, vs. 13.7-fold in HC+DFB; Table S2) might be mediated by the dissolution of particulate Fe by low pH or by the presence of strong organic chelators as observed in this very experiment (Segovia et al. 2017 and references therein). where dFe notably increased in treatments with high CO_2 and/or the addition of DFB (Figure 1). Furthermore, the dissolution of particulate Fe in the treatments with high CO_2 and/or the addition of DFB was evident in the Fe partitioning coefficients—the molar ratio between particulate and dissolved concentrations. On day 21, the Fe partitioning coefficients varied by 22-fold between the highest for the control (LC-DFB: 1.039) and lowest for the HC+DFB treatments (HC+DFB: 0.047; Figure S2). Thus, either the DFB addition or high CO_2 promoted the dissolution of pFe. However, at the end of the experiment, high dFe concentrations were only observed in the treatments with the DFB additions, suggesting that the presence of strong organic Fe chelators, such as DFB, mediated the maintenance of high dissolved Fe concentrations, as previously observed (Segovia et al. 2017). Thus, in our future oceans, high CO_2 (low pH) will increase dissolved Fe concentrations in regions rich in particulate Fe, and in strong organic Fe chelators. The deleterious effects of OA on the development of ecologically important species sensitive to increased CO_2 such as *E. Huxleyi*, will be more relevant in high-Fe environments than in Fe-limited ones.

In contrast to the findings for Fe, particulate Cu concentrations a) were not affected by either high CO_2 or the DFB addition; b) were dominated by a biogenic component and c) were significantly correlated with phytoplankton biomass (Table 4 and 5). Furthermore, unique to Cu was a significant increase in Cu:P ratios by day 21 in the high CO_2 treatments, especially when no DFB was added. Since the Cu partitioning coefficients only varied by 3.25 fold among treatments on day 21 (LC-DFB: 0.065 vs. HC+DFB: 0.047; data not shown), we hypothesize that high CO_2 did not affect the partitioning between particulate and dissolved Cu, but instead, it affected the speciation of dissolved Cu, increasing its bioavailability. This resulted in the highest Cu:P ratios in the high CO_2 treatments, despite the low phytoplankton biomass. This increase in bioavailability under lower pH is typical of metals that form strong inorganic complexes with carbonates, such as Cu^{2+} (Millero et al., 2009). Thus in our future oceans, high CO_2 (low pH) will shift the speciation of dissolved Cu towards higher abundance of free ionic species, increasing its bioavailability and likely its toxicity.

Similarly to Cu, particulate Co, Zn and Mn were correlated with biomass and were dominated by the biogenic component. But in contrast to Cu, these metals particulate concentrations were affect by increased CO_2 and/or the DFB addition. However, the effects of high CO_2 and/or DFB were very complex because significant interactions between these 2 factors were observed (Table 1); and further studies are required before we are able to discern and conclude a significant trend.. Yet, the P-normalized ratios of Co, Zn and Mn were significantly affected by CO_2 (Table 3), exhibiting moderately lower ratios under high CO_2, when phytoplankton biomass was lowest. These results imply that the bioavailability of these metals was not enhanced under acidic conditions. This suggests that under high CO_2 (low pH) the free ionic species of these metals will not significantly increase in the future, as shown for metals that occur predominantly as free ionic species in seawater (Millero et al., 2009).

5. Concluding remarks

Eliminado: was Fe (

Con formato: Inglés (británico)

Eliminado: and Ti were the only metals with positive correlations with Al and without correlation with phytoplankton biomass (Figure 1, Table 5), indicating

Eliminado: . The similarity between our values Fe:P values and that of the crustal ratio (Figure 1) also supports this finding. Indeed, the Fe:P ratios were significantly higher than those of indigenous plankton assemblages

Con formato: Inglés (británico)

Con formato: Inglés (británico)

Con formato: Inglés (británico)

Con formato: Inglés (británico)

Eliminado: cultures (Figure 1). We believe

Con formato: Inglés (británico)

Eliminado: the lithogenic fraction of Fe in the bulk particles in our experiment masked the biogenic signal, as proposed by King et al. (2012). Interestingly, the particulate Fe …

Con formato: Inglés (británico)

Eliminado: (nmol L^{-1}) decreased between days 12 and 21 (Table 3 and 5); this could be due to the solubilisation of ... [72]

Con formato: Inglés (británico)

Eliminado: the increase in dissolved Fe

Con formato: Inglés (británico)

Eliminado: Table 1). The sinking of pFe could have ... [73]

Con formato: Inglés (británico)

Eliminado: dissolved phase (i.e. partition coefficient, ... [74]

Con formato ... [75]

Eliminado: Fe from the

Con formato ... [76]

Eliminado: to the soluble pool (Segovia et al., 2017). In ... [77]

Con formato ... [78]

Eliminado: our experiment.¶ ... [79]

Con formato ... [80]

Eliminado: **dissolved Fe levels on particulate metal** ... [81]

Movido hacia abajo[8]: 2009).

Con formato: Inglés (británico)

Eliminado: The decrease in particulate Fe might have been ... [82]

Con formato: Inglés (británico)

Eliminado: resulted in higher dissolved Fe, and optimal pH ... [83]

Con formato ... [84]

Eliminado: Me:P ratios

Con formato ... [85]

Eliminado: Cu, Co, Zn, and Mn. For example, while Zn:P ... [86]

Con formato ... [87]

Movido (inserción)[8]

Con formato: Inglés (británico)

Con formato: Inglés (británico)

The results presented here show that in the fjord where we carried out the present experiment, particulate Fe was dominated by lithogenic material, and was significantly decreased by future predicted CO$_2$ concentrations (HC, 900 µatm) and DFB addition. This condition may well be comparable to most coastal ecosystems in the future ocean. Indeed, high CO$_2$ and/or DFB promoted the dissolution of particulate Fe, and the presence of this strong organic complex helped maintaining high dissolved Fe. Under control conditions at present CO$_2$ concentration (LC, 380 µatm) and no DFB amendment, the globally important coccolithophore *Emiliania huxleyi* was experiencing Fe limitation (Segovia et al. 2017). The shift between particulate and dissolved Fe promoted a massive bloom of *E. huxleyi* in the treatments with ambient CO$_2$ due to increased Fe bioavailability (for further details on Fe-bioavailability in *E. huxleyi* please see Segovia et a. 2017). Moreover, the negative effects of high CO$_2$ were mitigated by enhanced dFe. During the mentioned bloom, the concentrations of particulate metals with a strong biogenic component (Cu, Co, Zn, Cd, Mn, and Mo) were a) highly dynamic, b) positively correlated with plankton biomass, and c) influenced by growth requirements. Furthermore, high CO$_2$ decreased the Me:P ratios of Co, Zn and Mn, mainly due to low phytoplankton biomass, while increased the Cu:P ratios. In contrast DFB had no effects on these ratios. According to our results, high CO$_2$ may decrease particulate Fe and increase dissolved Fe, but high concentrations of dissolved Fe will only be maintained by the presence of strong organic ligands. The decrease in particulate Fe may affect the sinking flux of other metals associated with terrestrial material/dust in open ocean settings. Furthermore, ocean acidification will decrease *E. huxleyi* abundance, and as a result, the concentration of many biogenic particulate metals. Moreover, the Me:P ratios of metals that are predominately present in an ionic free form in seawater (e.g. Co, Zn and Mn) will likely decrease or stay constant. However, the high pZn observed will possibly be the result of anthropogenic aerosols, and the responsible for the low pCd registered, most likely due to the antagonistic interaction between Zn and Cd. In contrast, high CO$_2$ is predicated to shift the speciation of dissolved metals associated with carbonates, such as Cu, increasing their bioavailability, and resulting in higher Me:P ratios. We suggest that high Cu might be putative in this fjord, and autochthonous plankton might be adapted to cope with high Cu levels by developing specific physiological mechanisms. Future predicted high CO$_2$ levels are expected to change the relative concentrations of particulate and dissolved metals, due to the differential effects of high CO$_2$ on trace metal solubility, speciation, adsorption and toxicity, as well as on the growth of different phytoplankton taxa, and their elemental trace metal composition. In the future ocean, this will have great implications in the carbon cycle and the biological pump, consequently affecting the physic, chemical and biological aspects, i.e. marine systems dynamics.

Eliminado: Knowledge of the trace metal composition of in situ marine phytoplankton may allow us to estimate how microalgae in the future will influence the relative distribution and vertical transport of trace metals in the ocean. The results presented here showed that except for Ti and Fe, the trace metal concentrations of marine particles during a bloom of *Emiliania huxleyi* were a) highly dynamic, b) positively correlated with plankton biomass, c) influenced by growth requirements, and d) strongly affected by changes in CO$_2$ and dissolved Fe. According to our results, ocean acidification will decrease *E. huxleyi* abundance, and as a result, the concentration of some particulate trace metals that are especially high in *E. huxleyi,* such as Zn. Most importantly, OA is expected to change the relative concentrations of particulate metals, due to the differential effects of OA on the growth of marine phytoplankton species, and the contrasting metal requirements of phytoplankton phyla. OA might also affect the sinking flux of particulate Fe which would have an impact on the sinking of particulate metals associated with terrestrial material/dust in open ocean settings. Therefore, as suggested by Twining and Baines (2013), we require the development of ecophysiological models that link trace element composition of phytoplankton to physiological performance, as well as ecological models that are able to predict plankton physiological strategies and metal composition in a changing ocean, and the resulting effects of phytoplankton on the biogeochemical cycles of metals under a rapidly changing ocean.¶

Con formato: Fuente: Sin Negrita, Inglés (británico)

Con formato: Inglés (británico)

Acknowledgments

This work was funded by CTM/MAR 2010-17216 (PHYTOSTRESS) research grant from the Spanish Ministry for Science and Innovation (Spain) to MS, and by NSERC grants (Canada) to MTM and JTC. MRL was funded by a FPU grant from the Ministry for Education (Spain) and by fellowships associated to the mentioned above research grants to carry out a short-stay at MTM and JTC laboratories to analyze dissolved and particulate metals. We thank all the participants of the PHYTOSTRESS experiment for their collaboration, and the MBS (Espegrend, Norway) staff for logistic support during the experiment.

Eliminado: analyse

Con formato: Inglés (británico)

References

Ahner, B.A., Liping, W., Oleson, J.R., Ogura, N.: Glutathione and other low molecular weight tiols in marine phytoplankton under metal stress, Mar. Ecol. Prog. Ser., 232, 93-103, 2002.

Behrenfeld, M.J., Milligan, A.J.: Photophysiological expressions of iron stress in phytoplankton, Ann. Rev. Mar. Sci. 5, 217–246, 2013.

Canadell, J.G., Quéré, C., Raupach, M.R., Field, C.B., Buitenhuis, E.T., Ciais, P., Conway, T.J., Gillet, N.P., Houghton, R.A., Marland, G.: Contributions to accelerating atmospheric CO$_2$ growth from economic activity, carbon intensity, and efficiency of natural sinks. Proc. Natl. Acad. Sci. USA 104, 18886-18870, 2007

Crawford, D.W., Lipsen, M.S., Purdie, D.A., Lohan, M.C., Statham, P.J., Whitney, F.A., Putland, J.N., Johnson, W.K., Sutherland, N., Peterson, T.D., Harrison, P.J., Wong, C.S.: Influence of zinc and iron enrichments on phytoplankton growth in the northeastern subarctic Pacific. Limnol. Oceanogr, 48,1583–1600, 2003.

Cullen, J.T., Sherrell, R.M.: Effects of dissolved carbon dioxide, zinc, and manganese on the cadmium to phosphorus ratio in natural phytoplankton assemblages, Limnol. Oceanogr. 50, 1193–1204, 2005.

Desboeufs, K.V. A. Sofikitis, R. Losno, J.L. Colin, P. Ausset. 2005. Dissolution and solubility of trace metals from natural and anthropogenic aerosol particulate matter Chemosphere 58 (2): 195-203 DOI: 10.1016/j.chemosphere.2004.02.025

Dymond, J., Lyle, M.: Flux comparisons between sediments and sediment traps in the eastern tropical Pacific: implications for atmospheric CO$_2$ variations during the Pleistocene. Limnol. Oceanogr, 30, 699–712, 1985

Field, C.B.: Primary production of the biosphere: Integrating terrestrial and oceanic components, Science 281, 237–240, 1998.

Frew, R.D., Hutchins, D.A., Nodder, S., Sanudo-Wilhelmy, S., Tovar-Sanchez, A., Leblanc, K., Hare, C.E., Boyd, P.W.: Particulate iron dynamics during Fe Cycle in subantarctic waters southeast of New Zealand, Glob. Biogeochem. Cycles 20, GB1S93. http://dx. doi.org/10.1029/2005GB002558, 2006.

Guo, J., Lapi, S., Ruth, T.J., Maldonado, M.T.: The effects of iron and copper availability on the copper stoichiometry of marine phytoplankton. J. Phycol, 48, 312–325, 2012.

Ho, T-Y.: The trace metal composition of marine microalgae in cultures and natural assemblages. In: Rao S (ed) Algal cultures, analogues of blooms and applications, Science Publishers, New Hampshire, p 271–299, 2006.

Ho, T., Quigg, A., Zoe, V., Milligan, A.J., Falkowski, P.G., Morel, M,M.: The elemental composition of some marine phytoplankton. J. Appl. Phycol 1159,1145–1159, 2003

Ho, T-Y., Wen, L-S., You, C-F., Lee, D-C.: The trace metal composition of size-fractionated plankton in the South China Sea: Biotic versus abiotic sources. Limnol. Oceanogr, 52, 1776–1788, 2007.

Ho, T-Y., You, C.F., Chou, W-C., Pai, S-C., Wen, L-S., Sheu, D.D.: Cadmium and phosphorus cycling in the water column of the South China Sea: The roles of biotic and abiotic particles, Mar. Chem. 115-125-133, 2009.

Hoffmann, L.J., Breitbarth, E., Boyd P.W., Hunter, K.A.: Influence of ocean warming and acidification on trace metal biogeochemistry, Mar Ecol Prog Ser 470-191–205, 2012.

Hutchins, D., Mulholland, M., Fu, F.: Nutrient cycles and marine microbes in a CO$_2$-enriched ocean, Oceanography 22-128–145, 2009.

Jakuba, R.W., Moffett, J.W., Dyhrman, S.T.: Evidence for the linked biogeochemical cycling of zinc, cobalt, and phosphorus in the western North Atlantic Ocean, Global Biogeochem. Cycles 22, GB4012, 2008.

King, A.L., Sanudo-Wilhelmy, S.A., Boyd, P.W., Twining, B.S., Wilhelm, S.W., Breene, C., Ellwood, M.J. and Hutchins, D.A.: A comparison of biogenic iron quotas during a diatom spring bloom using multiple approaches, Biogeosciences 9, 667–687, 2012.

Mackey, K.R.M., Morris, J.J., Morel, F.M.M.: Response of photosynthesis to ocean acidification, Oceanography 28,74–91, 2015.

Millero, F.J., Woosley, R., Ditrolio, B., Waters, J.: Effect of ocean acidification on the speciation of metals in seawater. Oceanography 22:72–85, 2009.

Morel, F.M., Price, N.M.: The biogeochemical cycles of trace metals in the oceans, Science 300, 944–947, 2003.

Muller, F.L., Larsen, A., Stedmon, C.A., Søndergaard, M.: Interactions between algal – bacterial populations and trace metals in fjord surface waters during a nutrient-stimulated summer bloom, Limnol Oceanogr 50, 1855–1871, 2005.

Con formato: Inglés (británico)

Paasche, E.: A review of the coccolithophorid *Emiliania huxleyi* (Pymnesiophyceae), with particular reference to growth, coccolith formation, and calcification-photosynthesis interactions, Phycologia 40, 503–529, 2002.

Price, N.M., Morel, F.M.M.: Cadmium and cobalt substitution for zinc in a marine diatom, Nature 344, 658–660, 1990.

Rauschenberg, S., Twining, B.S.: Evaluation of approaches to estimate biogenic particulate trace metals in the ocean, Mar Chem 171, 67-77, 2015

Riebesell, U., Tortell, P.D.: Effects of ocean acidification on pelagic organisms and ecosystems, In: Gatusso J-P, Lansson L (eds) Ocean Acidification. Oxford University Press., Oxford, p 99–121, 2011.

Robbins, L.L., Hansen, M.E., Kleypas, J.A., Meylan, S.C.: CO_2calc: A User Friendly Carbon Calculator foe Windows, Mac OS X and iOS (iPhone), Open File Rep. 2010-1280, 2010.

Sanudo-Wilhelmy, S.A., Tovar-Sanchez, A., Fu, F.X., Capone, D.G., Carpenter, E.J., Hutchins, D.A.: The impact of surface-adsorbed phosphorus on phytoplankton Redfield stoichiometry, Nature 432, 897–901, 2004.

Semeniuk, D.M, Bundy, R.M., Posacka, A.M., Robert, M., Barbeau K.A., Maldonado, M.T.: Using ^{67}Cu to study the biogeochemical cycling of copper in the northeast subarctic Pacific ocean, Frontiers Mar. Sci. 3, 2-19, 2016.

Segovia, M., Lorenzo, M.R., Maldonado, M.T., Larsen, A., Berger, S.A., Tsagaraki, T.M., Lázaro, F.J., Iñiguez, C., García- Gómez, C., Palma, A., Mausz, M.A., Gordillo, F.J.L., Fernández, J.A., Ray, J. L., Egge, J.K.: Iron availability modulates the effects of future CO_2 levels within the marine planktonic food web, Mar. Ecol. Progr. Ser. 565, 17–33, 2017.

Shi, D., Xu, Y., Hopkinson, B.M., Morel, F.M.M.: Effect of ocean acidification on iron availability to marine phytoplankton, Science 327, 676–679, 2010.

Stocker, T.F., Qin, D., Plattner, G-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds) IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovern- mental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2013.

Sunda, W.G., Huntsman, S.A.: Iron uptake and growth limitation in oceanic and coastal phytoplankton, Mar. Chem. 50, 189–206, 1995a.

Sunda, W.G., Huntsman, S.A.: Cobalt and zinc interreplacement in marine phytoplankton: biological and geochemical implications, Limnol. Oceanogr. 40, 1404–1417, 1995b.

Sunda, W.G., Huntsman, S.A. Control of Cd concentrations in a coastal diatom by interactions among free ionic Cd, Zn, and Mn in seawater, Environ Sci Technol 32:2961–2968, 1998.

Sunda, W.G., Huntsman, S.A.: Effect of Zn, Mn, and Fe on Cd accumulation in phytoplankton: implications for oceanic Cd cycling, Limnol. Oceanogr. 45, 1501–1516, 2000.

Sunda, W.G.: Feedback interactions between trace metal nutrients and phytoplankton in the ocean, Front Microbiol 3, 204, 2012.

Tang, D.G.,Morel, F.M.M.: Distinguishing between cellular and Fe-oxide-associated trace elements in phytoplankton, Mar. Chem. 98, 18–30, 2006.

Taylor, S.: Abundance of chemical elements in the continental crust: a new table. Geochim Cosmochim Acta 28, 1273–1285, 1964.

Tovar-Sanchez, A., Sanudo-Wilhelmy, S.A., Garcia-Vargas, M., Weaver, R.S., Popels, L.C., Hutchins, D.A.: A trace metal clean reagent to remove surface-bound iron from marine phytoplankton, Mar. Chem. 82, 91–99, 2003.

Tovar-Sanchez, A., Sanudo-Wilhelmy, S.A., Kustka, A.B., Agustí, S., Dachs, J., Hutchins, D.A., Capone, D.G. and Duarte, C.M.: Effects of dust deposition and river discharges on trace metal composition of Trichodesmium spp. in the tropical and subtropical North Atlantic Ocean, Limnol Oceanogr 51, 1755–61, 2006.

Twining, B.S., Baines, S.B.: The trace metal composition of marine phytoplankton, Ann Rev Mar Sci 5, 191–215,

Eliminado: Nuester, J., Vogt, S., Newville, M., Kustka, A.B., Twining, B.S.: The unique biogeochemical signature of the marine diazotroph *Trichodesmium*, Front Microbiol Chem 3,150, 2012.¶

Con formato: Inglés (británico)

2013.

Xu, Y., Tang, D., Shaked, Y., Morel, F.M.M.: Zinc, cadmium, and cobalt interreplacement and relative use efficiencies in the coccolithophore *Emiliania huxleyi*, Limnol Oceanogr 52, 2294–2305, 2007.

Zamzow, H., Coale, K.H., Johnson, K.S.,Sakamoto, C.M.: Determination of copper complexation in seawater using flow injection analysis with chemiluminescence detection, Anal Chim Acta 377, 133–144, 1998.

Zondervan, I.: The effects of light, macronutrients, trace metals and CO_2 on the production of calcium carbonate and organic carbon in coccolithophores–A review, Deep Sea Res Part II Top Stud Oceanogr 54,521–537, 2007.

Página 6: [1] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 6: [2] Con formato María Segovia 11/6/19 19:41:00

Ninguno, Agregar espacio entre párrafos del mismo estilo, Control de líneas viudas y huérfanas

Página 6: [3] Con formato María Segovia 11/6/19 19:41:00

Color de fuente: Texto 1, Inglés (británico)

Página 6: [4] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 6: [5] Con formato María Segovia 11/6/19 19:41:00

Fuente: 12 pto, Inglés (británico)

Página 6: [6] Eliminado María Segovia 11/6/19 19:41:00

Página 6: [7] Con formato María Segovia 11/6/19 19:41:00

Fuente: Negrita, Color de fuente: Texto 1, Inglés (británico)

Página 6: [8] Eliminado María Segovia 11/6/19 19:41:00

Página 6: [9] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 6: [10] Eliminado María Segovia 11/6/19 19:41:00

Página 6: [11] Con formato María Segovia 11/6/19 19:41:00

Color de fuente: Texto 1, Inglés (británico)

Página 6: [12] Con formato María Segovia 11/6/19 19:41:00

Color de fuente: Texto 1, Inglés (británico)

Página 6: [13] Eliminado María Segovia 11/6/19 19:41:00

Página 6: [14] Con formato María Segovia 11/6/19 19:41:00

Color de fuente: Texto 1, Inglés (británico)

Página 6: [15] Eliminado María Segovia 11/6/19 19:41:00

Página 6: [16] Con formato María Segovia 11/6/19 19:41:00

Color de fuente: Texto 1, Inglés (británico)

Página 6: [17] Eliminado María Segovia 11/6/19 19:41:00

Página 6: [18] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 6: [18] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 6: [19] Eliminado María Segovia 11/6/19 19:41:00

Página 6: [20] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 6: [21] Eliminado María Segovia 11/6/19 19:41:00

Página 6: [22] Con formato María Segovia 11/6/19 19:41:00

Color de fuente: Texto 1, Inglés (británico)

Página 6: [23] Con formato María Segovia 11/6/19 19:41:00

Color de fuente: Texto 1, Inglés (británico)

Página 6: [23] Con formato María Segovia 11/6/19 19:41:00

Color de fuente: Texto 1, Inglés (británico)

Página 6: [24] Con formato María Segovia 11/6/19 19:41:00

Color de fuente: Texto 1, Inglés (británico)

Página 6: [25] Con formato María Segovia 11/6/19 19:41:00

Color de fuente: Texto 1, Inglés (británico)

Página 6: [26] Con formato María Segovia 11/6/19 19:41:00

Color de fuente: Texto 1, Inglés (británico)

Página 6: [27] Con formato María Segovia 11/6/19 19:41:00

Color de fuente: Texto 1, Inglés (británico)

Página 6: [28] Con formato María Segovia 11/6/19 19:41:00

Color de fuente: Texto 1, Inglés (británico)

Página 6: [29] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 6: [30] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 6: [31] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 6: [31] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 6: [32] Con formato María Segovia 11/6/19 19:41:00

Color de fuente: Texto 1, Inglés (británico)

Página 6: [32] Con formato María Segovia 11/6/19 19:41:00

Color de fuente: Texto 1, Inglés (británico)

Página 6: [33] Eliminado María Segovia 11/6/19 19:41:00

Página 6: [34] Con formato **María Segovia** **11/6/19 19:41:00**

Inglés (británico)

Página 6: [35] Con formato **María Segovia** **11/6/19 19:41:00**

Inglés (británico)

Página 6: [36] Con formato **María Segovia** **11/6/19 19:41:00**

Nivel 1, No agregar espacio entre párrafos del mismo estilo, Sin control de líneas viudas ni huérfanas

Página 6: [37] Con formato **María Segovia** **11/6/19 19:41:00**

Inglés (británico)

Página 10: [38] Con formato **María Segovia** **11/6/19 19:41:00**

Inglés (británico)

Página 10: [39] Con formato **María Segovia** **11/6/19 19:41:00**

Inglés (británico)

Página 10: [40] Con formato **María Segovia** **11/6/19 19:41:00**

Inglés (británico)

Página 10: [41] Con formato **María Segovia** **11/6/19 19:41:00**

Inglés (británico)

Página 10: [42] Con formato **María Segovia** **11/6/19 19:41:00**

Inglés (británico)

Página 10: [43] Con formato **María Segovia** **11/6/19 19:41:00**

Inglés (británico)

Página 10: [44] Con formato **María Segovia** **11/6/19 19:41:00**

Inglés (británico)

Página 10: [45] Eliminado **María Segovia** **11/6/19 19:41:00**

Página 10: [46] Con formato **María Segovia** **11/6/19 19:41:00**

Inglés (británico)

Página 10: [47] Con formato **María Segovia** **11/6/19 19:41:00**

Inglés (británico)

Página 10: [48] Eliminado **María Segovia** **11/6/19 19:41:00**

Página 10: [49] Con formato **María Segovia** **11/6/19 19:41:00**

Inglés (británico)

Página 10: [50] Eliminado **María Segovia** **11/6/19 19:41:00**

Página 10: [51] Con formato **María Segovia** **11/6/19 19:41:00**

Inglés (británico)

Página 10: [52] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 10: [53] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 10: [54] Eliminado María Segovia 11/6/19 19:41:00

Página 10: [55] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 10: [56] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 10: [57] Eliminado María Segovia 11/6/19 19:41:00

Página 10: [58] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 10: [59] Eliminado María Segovia 11/6/19 19:41:00

Página 10: [60] Con formato María Segovia 11/6/19 19:41:00

Derecha: 0 cm, Agregar espacio entre párrafos del mismo estilo, Punto de tabulación: No en 11.75 cm

Página 10: [61] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 10: [62] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 10: [63] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 10: [64] Con formato María Segovia 11/6/19 19:41:00

Fuente: Cursiva, Inglés (británico)

Página 10: [65] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 10: [66] Eliminado María Segovia 11/6/19 19:41:00

Página 10: [67] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 10: [68] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 10: [69] Con formato María Segovia 11/6/19 19:41:00

Derecha: 0 cm, Espacio Después: 12 pto, Sin control de líneas viudas ni huérfanas, No ajustar espacio entre texto latino y asiático, No ajustar espacio entre texto asiático y números, Punto de tabulación: No en 11.75 cm

Página 10: [70] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 10: [71] Con formato María Segovia 11/6/19 19:41:00

Inglés (británico)

Página 11: [72] Eliminado María Segovia 11/6/19 19:41:00

Página 11: [73] Eliminado María Segovia 11/6/19 19:41:00

Página 11: [74] Eliminado María Segovia 11/6/19 19:41:00

Página 11: [75] Con formato María Segovia 11/6/19 19:41:00

Fuente de párrafo predeter., Fuente: 12 pto, Español

Página 11: [76] Con formato María Segovia 11/6/19 19:41:00

Fuente de párrafo predeter., Fuente: 12 pto, Español

Página 11: [77] Eliminado María Segovia 11/6/19 19:41:00

Página 11: [78] Con formato María Segovia 11/6/19 19:41:00

Fuente de párrafo predeter., Fuente: 12 pto, Español

Página 11: [79] Eliminado María Segovia 11/6/19 19:41:00

Página 11: [80] Con formato María Segovia 11/6/19 19:41:00

Fuente: Sin Negrita, Inglés (británico)

Página 11: [81] Eliminado María Segovia 11/6/19 19:41:00

Página 11: [82] Eliminado María Segovia 11/6/19 19:41:00

Página 11: [83] Eliminado María Segovia 11/6/19 19:41:00

Página 11: [84] Con formato **María Segovia** **11/6/19 19:41:00**

Fuente: Times, Color de fuente: Color personalizado(RGB(21;21;24)), Inglés (británico)

Página 11: [85] Con formato **María Segovia** **11/6/19 19:41:00**

Fuente: Times, Color de fuente: Color personalizado(RGB(21;21;24)), Inglés (británico)

Página 11: [86] Eliminado **María Segovia** **11/6/19 19:41:00**

Página 11: [87] Con formato **María Segovia** **11/6/19 19:41:00**

Fuente: Times, 12 pto, Inglés (británico)

Table 1. Statistical analyses (Split-plot ANOVA) of the effects of high CO_2, the addition of DFB, and their interaction, as well as the effect of time, on the concentrations of particulate metals (mmol L^{-1}, data in Table S2, and Figure 3) in particles collected from the different mesocosms treatments.

Factor	**Al**	**Ti**	**P**	**Fe**	**Cu**	**Co**	**Zn**	**Cd**	**Mn**	**Mo**	**Pb**
CO_2	ns	ns	**	*	ns	**	***	***	**	***	ns
DFB	ns	ns	*	ns	ns	*	**	ns	*	*	ns
CO_2 x DFB	ns	*	**	ns	ns	*	**	*	**	**	ns
Time	ns	ns	ns	***	*	***	***	***	***	***	**

*ns: not significant; * p <0.05; ** p <0.01; *** p<0.001*

Eliminado: **Supplemental Table S1.** Instrumental conditions of ICP-MS and measurement parameters used during determination of trace elements concentrations. ¶
Instrument conditions ... [1]

Eliminado: mesocosm

Table 2. The average metal ratios in the particles collected in this study (without oxalate wash) using the data reported in Table S2. The P-normalized ratios (mmol : mol P, Figure 4) are compared to previous estimates in marine plankton samples and phytoplankton cultures (A). The Al-normalized ratios (mmol : mol Al) are compared to crustal ratios (B).

A)

(mmol : mol P)	**Mn:P**	**Fe:P**	**Co:P**	**Cu:P**	**Zn:P**	**Cd:P**	**Mo:P**	**Al:P**	**Reference**
Phytoplankton $_{\text{Lab}}$	3.8	7.5	0.19	0.38	0.8	0.21	0.03		Ho et al. 2003
Marine Plankton $_{\text{Field}}$	0.68±0.54	5.1±1.6	0.15±0.06	0.41±0.16	2.1±0.88				Ho 2006
E. huxleyi $_{\text{Lab}}$	7.1±0.36	3.5±0.07	0.29±0.02	0.07±0.013	0.38±0.002	0.36±0.01	0.022±0.0003		Ho et al. 2003
This study	1.65±0.41	39.2±34.3	0.07±0.02	1.41±0.55	34.02±18.05	0.02±0.01	0.42±0.12	70±38	
Crust ratio	510	29,738	13	25	32	0.05	0.46	89,972	Taylor 1964

B)

(mmol : mol Al)	**Mn:Al**	**Fe:Al**	**Co:Al**	**Cu:Al**	**Zn:Al**	**Cd:Al**	**Mo:Al**	**Pb:Al**	**Ti:Al**
Crustal ratio	5.7	331	0.14	0.27	0.35	0.001	0.005	0.02	39
This study	35±28	506±342	1.5±1.2	26.5±15	795±865	0.5±0.4	8.6±6.5	4.9±3.9	119±47.6

Table 3. Statistical analyses (Split-plot ANOVA) of the effects of CO_2, DFB, and their interaction, as well as the effect of time, on the P-normalized metal quotas (mmol: mol P, data in Figure 4, and Table S2) in particles collected from the different mesocosm treatments.

Factor	**Fe:P**	**Cu:P**	**Co:P**	**Zn:P**	**Cd:P**	**Mn:P**	**Mo:P**	**Pb:P**	**Ti:P**
CO_2	ns	*	***	**	ns	*	ns	ns	ns
DFB	ns	ns	ns	ns	ns	ns	ns	ns	ns
CO_2 x DFB	ns	ns	ns	ns	ns	ns	ns	ns	ns
Time	***	***	***	***	ns	ns	ns	ns	***

*ns: not significant; * p <0.05; ** p <0.01; *** p<0.001*

Con formato: Fuente: +Títulos (Calibri), 10 pto, Negrita

Con formato: Derecha, Punto de tabulación: 15.5 cm, Derecha + No en 15 cm

Eliminado: ¶

Con formato: Espacio Después: 10 pto, Control de líneas viudas y huérfanas, Ajustar espacio entre texto latino y asiático, Ajustar espacio entre texto asiático y números

Table 4. The relationship (Pearson correlations, $p< 0.05$) between particulate metals concentrations (nmol L^{-1}, no oxalate wash, reported in Table S2) and the biomass (µgC L^{-1}) of *Emiliania huxleyi* and total cells (phytoplankton and microzooplankton) collected from the different mesocosm treatments.

		P	**Fe**	**Cu**	**Co**	**Zn**	**Cd**	**Mn**	**Mo**	**Pb**	**Ti**
E. huxleyi	Correlation coefficient	0.622	ns	0.614	0.756	0.747	0.818	0.686	0.825	ns	ns
	P-value	0.003		0.003	$7.35 \cdot 10^{-5}$	$1.01 \cdot 10^{-4}$	$6.02 \cdot 10^{-6}$	$5.93 \cdot 10^{-4}$	$4.20 \cdot 10^{-6}$		
Total cells	Correlation coefficient	0.641	ns	0.51	0.644	0.889	0.802	0.598	0.53	ns	ns
	P-value	0.002		0.02	$1.62 \cdot 10^{-3}$	$7.03 \cdot 10^{-8}$	$1.23 \cdot 10^{-5}$	$4.18 \cdot 10^{-3}$	$1.35 \cdot 10^{-2}$		

Con formato: Fuente: +Títulos (Calibri), 10 pto, Negrita

Con formato: Sangría: Izquierda: -1.25 cm

Comentado [mt4]: this table needs to be fixed. I removed the oxalate washed data...but the lines are still there...

Con formato: Fuente: 8 pto, Negrita, Inglés (británico)

Eliminado: ¶ ... [2]

Página 1: [1] Eliminado **María Segovia** **11/6/19 19:50:00**

Página 4: [2] Eliminado **María Segovia** **11/6/19 19:50:00**

Supplemental Figures

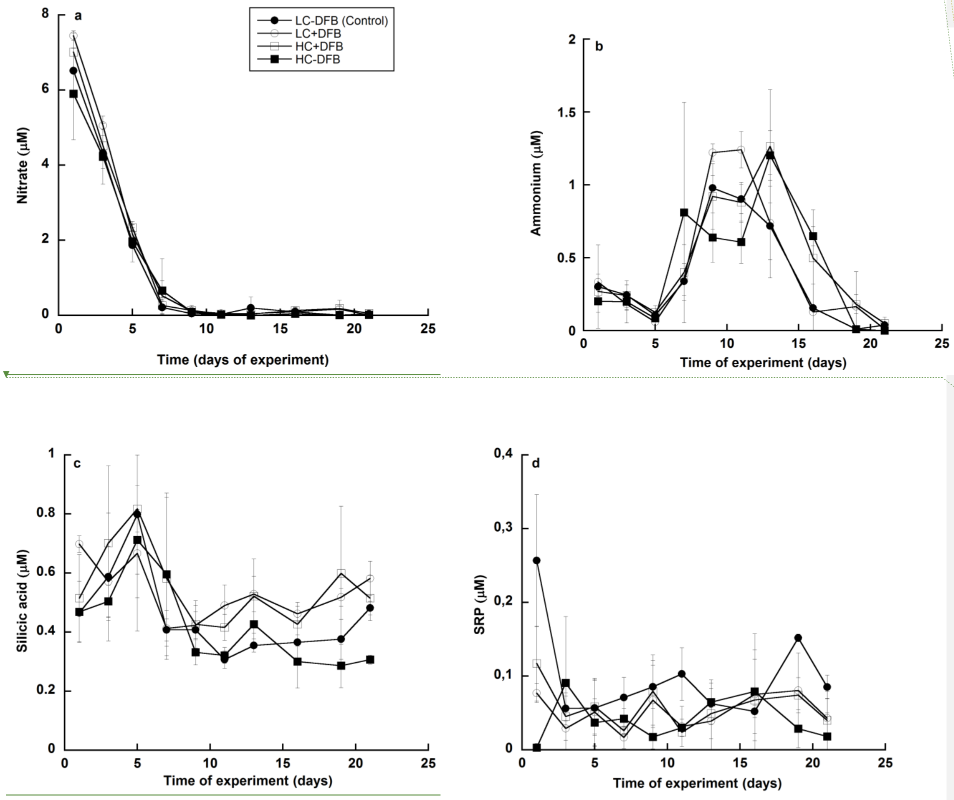
Particulate trace metal dynamics in response to increased CO_2 and iron availability in a coastal mesocosm experiment

M. Rosario Lorenzo[1], María Segovia[1], Jay T. Cullen[2], and María T. Maldonado[3]

Correspondence to: María Segovia (segovia@uma.es) and María T. Maldonado (mmaldonado@eoas.ubc.ca)

Con formato: Numeración: Continua

Supplemental Figure S1. Temporal development of major nutrient concentrations within the mesocosms in the different treatments (LC: ambient CO_2 (390 µatm); HC: increased CO_2 (900 µatm); -DFB: no DFB addition; +DFB: with a 70 nM DFB addition): (a) nitrate, (b) ammonium, (c) silicic acid, (d) soluble reactive phosphate (SRP).



Eliminado: *<object>*¶
¶
¶
¶

Con formato: Nivel 1, Derecha: 0.12 cm, No agregar espacio entre párrafos del mismo estilo, Punto de tabulación: 11.75 cm, Izquierda

Con formato: Fuente: 10 pto

Comentado [mt1]: the numbers in the Y-axis of the SRP graph need to be changed from 1,5 to 1.5 (please change the commas to dots).....

Eliminado: Salto de página

Eliminado: ¶
¶
<object>¶
¶
¶

Con formato: Agregar espacio entre párrafos del mismo estilo

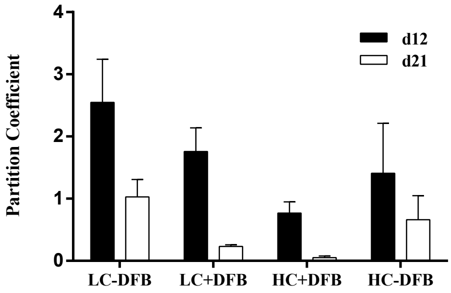
Con formato: Fuente: 12 pto, Sin Negrita

Con formato: Español

Con formato: Nivel 1, Derecha: 0.12 cm, Punto de tabulación: 11.75 cm, Izquierda

Supplemental Figure S2. The Fe partition coefficients (the molar ratio between particulate and dissolved concentrations) in the different mesocosm treatments; LC: ambient CO_2 (390 µatm); HC: increased CO_2 (900 µatm); -DFB: no DFB addition; +DFB: with a 70 nM DFB addition; on day 12 and day 21.

Con formato: Fuente: 10 pto



105

Supplemental Tables

Particulate trace metal dynamics in response to increased CO_2 and iron availability in a coastal mesocosm experiment

M. Rosario Lorenzo[1], María Segovia[1], Jay T. Cullen[2], and María T. Maldonado[3]

Correspondence to: María Segovia (segovia@uma.es) and María T. Maldonado (mmaldonado@eos.ubc.ca)

Supplemental Table S1. Instrumental conditions of ICP-MS and measurement parameters used during determination of trace elements concentrations.

Instrument conditions	
Instrument type	ELEMENT XR
Torch	Fassel type
Spray chamber	Glass cyclonic spray chamber
Nebuliser	ESI microflow ST nebuliser (selfaspirating)
Cones	Standard Ni sampler and skimmer
RF Power (W)	1120
Cooling gas flow rate ($L\ min^{-1}$)	16

Definición de estilo: Normal

Definición de estilo: Revisión

Eliminado: **Supplemental Table S1.** Instrumental conditions of ICP-MS and measurement parameters used during determination of trace elements concentrations. ¶

Auxiliary gas flow rate (L min^{-1})	0.9	
Sample gas flow rate (L min^{-1})	1.2	
Sample matrix	1% nitric acid	

Method acquisition parameters

Scan type	E-scan	
Spectral resolution	Low (nominal m/Δm~300)	Medium (nominal m/Δm~3000)
Isotopes of interes	^{95}Mo ^{98}Mo ^{111}Cd ^{114}Cd ^{206}Pb ^{208}Pb	^{27}Al ^{31}P ^{47}Ti ^{49}Ti ^{55}Mn ^{56}Fe ^{59}Co ^{63}Cu ^{65}Cu ^{6}6Zn ^{68}Zn
Internal standard	^{115}In	^{115}In
Mass window (%)	40	125
Samples/peak	10	20
Samples time (ms)	10	10
Runs	3	3
Passes	10	10

Supplemental Table S2. The concentration of particulate metals (without oxalate wash) in seawater (nM) in the different mesocosm treatments (LC: ambient CO_2; HC: increased CO_2 (900 µatm); -DFB: no DFB addition; +DFB: with a 70 nM DFB addition) during the development of a bloom of *Emiliania huxleyi* (especially in treatment LC+DFB). Data are means of measurements in 3 independent mesocosms (n = 3) except for LC−DFB where n = 2. Error bars indicate SD. Data is plotted in Figure 3. Note that the concentrations of Co, Pb and Cd were much lower than the rest of the metals, so their values were multiplied by either (10) or (100) in the table (e.g. Co, Cd, and Pb concentrations on d12 in LC-DFB were 0.01, 0.0028 and 0.025 nmol L^{-1}, respectively).

	Treatment	**Al**	**Ti**	**P**	**Fe**	**Cu**	**Co (·10)**	**Zn**	**Cd (·100)**	**Mn**	**Mo**	**Pb (·10)**
d12												
	LC-DFB	9.16 (3.16)	1.30 (0.27)	131.8 (27.05)	13.5 (0.88)	0.24 (0.04)	0.10 (0.06)	3.24 (0.15)	0.28 (0.06)	0.24 (0.06)	0.08 (0.01)	0.25 (0.01)
	LC+DFB	29.2 (6.00)	3.16 (0.52)	329.3 (107.8)	14.8 (1.78)	0.30 (0.01)	0.22 (0.05)	14.81 (2.69)	0.91 (0.22)	0.54 (0.14)	0.09 (0.02)	0.56 (0.05)
	HC+DFB	11.0 (7.04)	1.23 (0.54)	120.1 (45.49)	7.29 (0.41)	0.32 (0.09)	0.07 (0.01)	3.13 (0.55)	0.26 (0.23)	0.17 (0.06)	0.04 (0.02)	0.12 (0.05)
	HC-DFB	18.1 (8.53)	1.28 (0.53)	193.7 (66.43)	11.2 (4.43)	0.29 (0.08)	0.11 (0.07)	4.48 (0.38)	0.23 (0.03)	0.29 (0.13)	0.07 (0.01)	0.85 (0.51)
d17												
	LC-DFB	27.1 (14.8)	0.27 (0.14)	171.6 (20.1)	17.1 (8.08)	0.10 (0.04)	0.07 (0.00)	2.87 (1.23)	0.45 (0.32)	0.20 (0.04)	0.08 (0.05)	0.28 (0.11)
	LC+DFB	29.2 (19.2)	4.63 (2.84)	972.8 (563)	12.2 (9.14)	1.02 (0.56)	0.68 (0.42)	62.7 (38.2)	2.38 (0.87)	2.36 (1.49)	0.37 (0.08)	0.77 (0.41)
	HC+DFB	5.94 (4.38)	0.59 (0.34)	134.1 (47.7)	1.98 (0.76)	0.13 (0.07)	0.05 (0.02)	2.53 (0.49)	0.19 (0.03)	0.14 (0.04)	0.06 (0.03)	0.14 (0.05)
	HC-DFB	35.4 (17.9)	4.11 (1.86)	372.7 (253)	9.34 (7.29)	0.50 (0.06)	0.19 (0.02)	5.88 (3.78)	0.98 (0.65)	0.56 (0.42)	0.09 (0.06)	1.42 (0.37)
d21												
	LC-DFB	19.2 (1.01)	2.95 (0.06)	341.9 (20.1)	5.83 (1.81)	0.48 (0.02)	0.35 (0.03)	15.5 (0.97)	1.13 (0.26)	0.66 (0.06)	0.10 (0.02)	2.07 (0.26)
	LC+DFB	9.18 (5.35)	1.53 (0.55)	380.9 (45.3)	2.52 (0.35)	0.44 (0.06)	0.37 (0.07)	26.2 (2.96)	1.41 (0.25)	0.88 (0.09)	0.20 (0.05)	1.23 (0.75)
	HC+DFB	2.64 (1.58)	0.49 (0.40)	95.9 (12.5)	0.53 (0.32)	0.15 (0.06)	0.09 (0.04)	3.24 (1.96)	0.30 (0.16)	0.14 (0.05)	0.05 (0.01)	0.19 (0.05)

HC-DFB	8.22 (2.05)	0.87 (0.20)	134.7 (22.1)	3.19 (1.21)	0.26 (0.05)	0.12 (0.02)	3.47 (0.97)	0.27 (0.13)	0.22 (0.08)	0.08 (0.03)	0.58 (0.18)

Supplemental Table S3. The concentration of particulate metals (with oxalate wash) in seawater (nmol L^{-1}) in the different mesocosm treatments; (LC: ambient CO_2; HC: increased CO_2 (900 µatm); -DFB: no DFB addition; +DFB: with a 70 nM DFB addition) during the development of a bloom of *Emiliania huxleyi* (especially in treatment LC+DFB). Data are means of measurements in 3 independent mesocosms (n = 3) except for LC−DFB where n = 2. Error bars indicate SD. Note that the concentrations of Co, Pb and Cd were much lower than the rest of the metals, so their values were multiplied by either (10) or (100) in the table (e.g. Co, Cd, and Pb concentrations on d12 in LC-DFB were 0.007, 0.0009 and 0.01 nmol L^{-1}, respectively). The percentage (%) indicates the mean quantity of metal remaining after the oxalate wash. Statistically significant differences are indicated with asterisk (* if p <0.05; ** if p <0.01 and *** if p<0.001; ns: not significant).

	Treatment	**Al**	**Ti**	**P**	**Fe**	**Cu**	**Co (·10)**	**Zn**	**Cd (·100)**	**Mn**	**Mo**	**Pb (·10)**
d12												
	LC-DFB	11.6 (2.8)	1.32 (0.34)	117 (3.27)	12.52 (0.78)	0.16 (0.03)	0.07 (0.00)	1.92 (0.86)	0.09 (0.06)	0.15 (0.02)	0.02 (0.00)	0.10 (0.00)
	LC+DFB	28.3 (12)	4.49 (1.91)	258 (46.1)	14.67 (3.35)	0.23 (0.08)	0.19 (0.00)	7.16 (1.29)	0.51 (0.14)	0.41 (0.06)	0.03 (0.01)	0.20 (0.11)
	HC+DFB	15.9 (2.3)	2.52 (0.66)	139 (14.2)	8.05 (1.08)	0.22 (0.06)	0.09 (0.01)	2.39 (0.93)	0.20 (0.09)	0.21 (0.07)	0.03 (0.01)	0.11 (0.06)
	HC-DFB	11.6 (8.8)	1.66 (0.68)	178 (66.3)	9.79 (3.75)	0.19 (0.08)	0.08 (0.03)	2.84 (0.52)	0.22 (0.06)	0.28 (0.08)	0.02 (0.01)	0.19 (0.08)
d17												
	LC-DFB	6.42 (2.9)	0.85 (0.35)	97 (41.6)	1.23 (0.56)	0.11 (0.07)	0.09 (0.05)	2.86 (1.45)	0.26 (0.09)	0.18 (0.07)	0.03 (0.00)	0.09 (0.05)
	LC+DFB	7.53 (4.7)	1.85 (0.63)	245 (136)	1.28 (0.68)	0.24 (0.11)	0.22 (0.08)	12.1 (3.78)	1.20 (0.69)	0.54 (0.29)	0.05 (0.03)	0.18 (0.09)
	HC+DFB	4.48 (0.2)	1.29 (0.01)	131 (5.31)	1.55 (0.19)	0.14 (0.01)	0.07 (0.00)	3.03 (0.90)	0.21 (0.06)	0.14 (0.02)	0.03 (0.00)	0.10 (0.05)
	HC-DFB	12.8 (2.7)	1.98 (0.74)	233 (162)	5.31 (0.99)	0.29 (0.11)	0.18 (0.06)	5.03 (3.06)	0.35 (0.13)	0.43 (0.33)	0.06 (0.01	
d21												
	LC-DFB	13.9 (3.2)	1.54 (0.48)	257 (20.9)	3.76 (0.75)	0.29 (0.06)	0.26 (0.01)	8.59 (0.69)	0.74 (0.31)	0.35 (0.04)	0.05 (0.02	
	LC+DFB	4.36 (0.4)	1.01 (0.41)	253 (47.6)	2.04 (0.63)	0.23 (0.02)	0.20 (0.01)	14.3 (1.32)	0.67 (0.09)	0.43 (0.05)	0.05 (0.01	
	HC+DFB	2.49 (0.9)	0.62 (0.17)	79 (19.6)	0.33 (0.07)	0.11 (0.02)	0.07 (0.03)	2.36 (1.38)	0.09 (0.06)	0.09 (0.03)	0.01 (0.00	
	HC-DFB	2.56 (1.2)	0.98 (0.30)	74 (20.7)	1.03 (0.18)	0.12 (0.03)	0.05 (0.01)	1.01 (0.35)	0.05 (0.02)	0.07 (0.01)	0.02 (0.00	
%		ns	ns	80*	75*	60*	70*	55**	45***	55**	35***	

Movido hacia abajo[1]: ¶
Salto de página
¶
Supplemental Table

Eliminado: Instrument conditions ... [1]

Movido (inserción)[1]

Celdas eliminadas

Celdas eliminadas ... [10]

Celdas insertadas ... [2]

Con formato: Fuente: Sin Negrita

Con formato ... [9]

Con formato: Fuente: 10 pto, Negrita

Tabla con formato

Celdas eliminadas

Celdas eliminadas ... [3]

Con formato: Fuente: 10 pto

Con formato ... [4]

Con formato ... [8]

Celdas insertadas

Con formato ... [5]

Celdas eliminadas ... [6]

Con formato: Espacio Después: 0 pto, No agregar espacio entre párrafos del mismo estilo, Control de líneas viudas y huérfanas, Ajustar espacio entre texto latino y asiático, Ajustar espacio entre texto asiático y números

Con formato: Izquierda: 3 cm, Derecha: 3 cm, Arriba: 2.5 cm, Abajo: 2.5 cm, Ancho: 20.99 cm, Alto: 29.7 cm, Distancia del encabezado desde el borde: 1.25 cm, Numeración: Continua

Con formato: Fuente: Sin Cursiva, Español

Página 5: [1] Eliminado Maria Segovia 12/6/19 12:33:00

Página 5: [2] Celdas insertadas María Segovia 11/6/19 20:05:00

Celdas insertadas

Página 5: [2] Celdas insertadas María Segovia 11/6/19 20:05:00

Celdas insertadas

Página 5: [2] Celdas insertadas María Segovia 11/6/19 20:05:00

Celdas insertadas

Página 5: [2] Celdas insertadas María Segovia 11/6/19 20:05:00

Celdas insertadas

Página 5: [2] Celdas insertadas María Segovia 11/6/19 20:05:00

Celdas insertadas

Página 5: [2] Celdas insertadas María Segovia 11/6/19 20:05:00

Celdas insertadas

Página 5: [2] Celdas insertadas María Segovia 11/6/19 20:05:00

Celdas insertadas

Página 5: [2] Celdas insertadas María Segovia 11/6/19 20:05:00

Celdas insertadas

Página 5: [2] Celdas insertadas María Segovia 11/6/19 20:05:00

Celdas insertadas

Página 5: [3] Celdas eliminadas María Segovia 11/6/19 20:05:00

Celdas eliminadas

Página 5: [3] Celdas eliminadas María Segovia 11/6/19 20:05:00

Celdas eliminadas

Página 5: [3] Celdas eliminadas María Segovia 11/6/19 20:05:00

Celdas eliminadas

Página 5: [4] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto

Página 5: [4] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto

Página 5: [4] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto

Página 5: [4] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto

Página 5: [5] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva

Página 5: [5] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva

Página 5: [6] Celdas eliminadas María Segovia 11/6/19 20:05:00

Celdas eliminadas

Página 5: [6] Celdas eliminadas María Segovia 11/6/19 20:05:00

Celdas eliminadas

Página 5: [6] Celdas eliminadas María Segovia 11/6/19 20:05:00

Celdas eliminadas

Página 5: [7] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto

Página 5: [7] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto

Página 5: [7] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto

Página 5: [7] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto

Página 5: [7] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto

Página 5: [7] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto

Página 5: [7] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto

Página 5: [7] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [7] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [7] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [8] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto

Página 5: [9] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto, Sin Cursiva

Página 5: [9] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto, Sin Cursiva

Página 5: [10] Celdas eliminadas **María Segovia** **11/6/19 20:05:00**

Celdas eliminadas

Página 5: [10] Celdas eliminadas **María Segovia** **11/6/19 20:05:00**

Celdas eliminadas

Página 5: [10] Celdas eliminadas **María Segovia** **11/6/19 20:05:00**

Celdas eliminadas

Página 5: [10] Celdas eliminadas **María Segovia** **11/6/19 20:05:00**

Celdas eliminadas

Página 5: [10] Celdas eliminadas **María Segovia** **11/6/19 20:05:00**

Celdas eliminadas

Página 5: [10] Celdas eliminadas **María Segovia** **11/6/19 20:05:00**

Celdas eliminadas

Página 5: [10] Celdas eliminadas **María Segovia** **11/6/19 20:05:00**

Celdas eliminadas

Página 5: [10] Celdas eliminadas María Segovia 11/6/19 20:05:00

Celdas eliminadas

Página 5: [10] Celdas eliminadas María Segovia 11/6/19 20:05:00

Celdas eliminadas

Página 5: [10] Celdas eliminadas María Segovia 11/6/19 20:05:00

Celdas eliminadas

Página 5: [10] Celdas eliminadas María Segovia 11/6/19 20:05:00

Celdas eliminadas

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato Maria Segovia 11/6/19 20:05:00

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro

Página 5: [11] Con formato **Maria Segovia** **11/6/19 20:05:00**

Fuente: 10 pto, Sin Cursiva, Color de fuente: Negro