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Influence of the Amazon River on dissolved and intra-cellular metal concentrations in *Trichodesmium* colonies along the western boundary of the sub-tropical North Atlantic Ocean

A. Tovar-Sanchez[1] and S. A. Sañudo-Wilhelmy[2]

[1]Department of Global Change Research, IMEDEA (CSIC-UIB) Instituto Mediterráneo de Estudios Avanzados, Miquel Marqués 21, 07190 Esporles, Spain
[2]University of Southern California, Department of Biological Sciences and Department of Earth Sciences, 3616 Trousdale Parkway, AHF 206, Los Angeles, CA90089-0371, USA

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Correspondence to: A. Tovar-Sanchez (atovar@imedea.uib-csic.es)

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Abstract

Despite the ecological importance of *Trichodesmium* spp. for the global oceanic nitrogen budget, there is limited information on their trace metal composition in field samples. We report dissolved (<0.22 µm) metal concentrations measured in surface waters (Ag, Cd, Co, Cu, Fe, Mo, Ni, P, Pb and V) and in the total and the intracellular pool (Ag, Al, Cd, Co, Cu, Fe, Mn, Mo, Ni, P, Pb, V) of *Trichodesmium* populations collected in the western subtropical North Atlantic Ocean (April–May 2003) within the influence of the Amazon River plume. Dissolved element distributions were strongly influenced by the River discharge, with concentrations of some elements varying directly (i.e. Cd, Mo and V) or inversely (Ag, Co, Cu, Fe, Ni, P and Pb) with surface salinity. Intracellular metal values to phosphorous ratios (mol:mol) for Cd, Co, Cu, Fe, Mn, Mo, Ni and V ranged from 9.0×10^{-6} for Cd to 4.4×10^{-2} for Fe. Although total metal composition was significantly correlated with the intracellular content in the *Trichodesmium* colonies for some elements (e.g., Co, Cu, V), metal pools in the phytoplankton did not co-vary with the dissolved metal concentrations, suggesting that water column measurements may not be good predictors of the intracellular metal concentrations. The impact of physical parameters and bioactive elements on biological processes in *Trichodesmium* such as nitrogen fixation, carbon drawdown and biomass production was explored by using a principal component analysis test (PCA). The analysis indicates that the biological drawdown of dissolved inorganic carbon (DIC) by *Trichodesmium* seems to be influenced by the internal content of Fe, Co, Cd, Cu and Mn, while nitrogen fixation seems more influenced by the internal concentration of Mo, Ni and V and by the dissolved phosphorous concentrations.

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1 Introduction

Marine cyanobacteria in the genus *Trichodesmium* are important N_2-fixing organisms in oligotrophic tropical and subtropical oceans, playing an important role in primary production and in the new nitrogen budget (Karl et al., 1997; Capone et al., 1997; LaRoche and Breitbarth, 2005; Westberry and Siegel, 2006). Therefore, important advances have been made in understanding their geographical distribution and abundance (e.g., Carpenter et al., 2004; Westberry and Siegel, 2006), nutrient requirements (Mills et al., 2004; Tuit et al., 2004; Kustka et al., 2002; Sañudo-Wilhelmy et al., 2001) and the effect of environmental variables on bloom dynamics (Subramaniam et al., 2008; Tovar-Sanchez et al., 2006). However, some critical issues still remain largely unknown, such as evaluating the impact of the Amazon River on the cycling of bioactive dissolved metals, and the impact of that river on intracellular metal levels in field populations of *Trichodesmium*. Whereas total metal composition of field-collected *Trichodesmium* colonies have been reported (Tovar-Sanchez et al., 2006), their internal metal pool and its relation to both the Amazon River plume and bloom dynamics are still unknown. The internal metal pool represents the biological fraction (e.g., cellular quota, Hassler and Schoemann, 2009; Tovar-Sanchez et al., 2003), and therefore quantification of this metal pool is important to understand the biochemical status and/or the nutritional requirements of *Trichodemium* in the field. Furthermore, simultaneous measurements of bioactive trace metals in the intracellular and in the dissolved water column pools could help us to better understand the processes and mechanisms influencing the bioavailability of trace elements in the field.

In this study, we investigated the geographical distribution of dissolved elements in surface waters both in and out of the influence of the Amazon River plume, and the relationship of the plume with the internal metal composition of *Trichodesmium*. A Principal Component Analysis (PCA) was carried out to try to identify the bioactive trace elements and physical parameters (e.g., salinity, temperature, and mixed layer depth) influencing nitrogen fixation, carbon drawdown and biomass production during our study along the western boundary of the subtropical Atlantic Ocean.

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2 Methods

2.1 Sampling and analysis

Sampling was carried out during April–May 2003 under high riverine flow conditions (DeMaster and Pope, 1996). A total of 42 locations, distributed along the western tropical North Atlantic Ocean within and outside of the influence of the Amazon River (Fig. 1), were sampled in this study. Dissolved surface seawater samples were collected from a "towed fish" deployed at 2 m below the surface and towed at about 5 knots during sampling. Seawater was pumped through acid-cleaned Teflon tubing coupled to a C-flex tubing (for the Cole-Parmer peristaltic pump head), filtered through an acid-cleaned polypropylene cartridge filter (0.22 µm, MSI, Calyx®), and collected in a 1 L LDPE acid-washed bottle. Seawater samples were acidified with sub-boiling quartz distilled HCl (Q-HCl) to pH <1.5 and stored for at least 1 month prior to analysis. Metal concentrations (Ag, Cd, Co, Cu, Fe, Mo, Ni, Pb, and V) were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS; ThermoFinigan, Element 2) after pre-concentration with an ammonium1-pyrrolidine-dithiocarbamate/diethylammonium diethyldithiocarbamate (APDC/DDDC) organic extraction (Bruland et al., 1985). Dissolved phosphorous concentrations were determined using the MAGIC method (Karl and Tien, 1992).

Trichodesmium colonies were collected at a depth of ~5 m using an acid-cleaned all-plastic 100-micron mesh plankton net. Individual colonies were removed from the acid-cleaned polyethylene net collector with a plastic inoculating loop in a class-100 laminar flow hood. Approximately 100 colonies were collected at each location and stored frozen in Teflon vials until acid digestion. Samples were digested in Teflon digestion vials using combined Q-HNO_3 (60%), Q-HCl (30%) and Q-HF (10%), and heated on a hot plate until complete digestion (Sañudo-Wilhelmy et al., 2001; 2004). Metal concentrations (Ag, Cd, Co, Cu, Fe, Mn, Mo, Ni, P and V) were determined by ICP-MS in the acid-digests. Intracellular metal levels were determined after washing a sub-sample of the field-collected *Trichodesmium* colonies with the oxalate reagent

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described by Tovar-Sanchez et al. (2003).

3 Results and discussion

3.1 Dissolved trace metal distributions

Dissolved metal (Ag, Cd, Co, Cu, Fe, Mo, Ni, Pb, V) and P concentrations measured in surface waters during our study are reported in Table S1 in the auxiliary material section. The impact of the Amazon River plume on the chemical composition of our sampling locations is reflected in the salinity gradient (22.4–36.3) as well as in the dissolved trace element distributions (Table S1). The influence of the river plume on metal distributions was different for different metals. For example, the spatial gradient in dissolved Ag, Co, Cu, Fe, Ni, Pb, and P (range of concentrations from low to high salinity: Ag, 15.50–8.43 pM; Co, 172.7–17.32 pM; Cu, 7.69–0.46 nM; Fe, 8.70–0.41 nM; Ni, 1.95–0.88 nM; Pb, 77.77–10.09 pM; P, 33.72–2.31 nM) varied inversely with salinity (Figs. 2a and S1a–f in the supplementary information), suggesting that the Amazon River plume was the main source of these elements to the area of study during our sampling.

In contrast, the spatial distribution of dissolved Cd, Mo and V (range from low to high salinity: Cd, 0.31–0.53 nM; Mo, 0.08–0.13 µm; V, 20.92–35.69 nM) varied directly with salinity, suggesting that the Amazon River plume is not the source of these elements, but rather the river input diluted their concentration in the Ocean (Figs. 2b and S1g–h in the supplementary information).

To further establish the impact of the Amazon River plume on trace element distributions in our area of study, we collected water samples at 6 stations located in and out of the plume in a 24 h period (salinity range: 22.4–30.9; stations 30–35 in Table S1). The positive or negative slopes obtained for different elements in the linear regression analysis with salinity confirms our results regarding the different influence of the river on metal concentrations discussed above (insets in Figs. 2 and S1 in the supplementary information). Although our data is very limited, we used those metal-salinity linear

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regressions to obtain a first-order approximation of the "hypothetical" metal concentrations at the low-salinity end-member of the Amazon River basin that would explain the concentrations measured at the seawater end member assuming conservative estuarine mixing (Table 1). Predicted metal concentrations at salinities 5 and 10 for Cd (0.10 and 0.16 nM), V (11.5 and 14.3 nM), Co (145.5 and 130.0 pM) and Ni (2.4 and 2.3 nM) were in good agreement with the concentrations measured by Seyler and Boaventura (2003) in the upper Amazon River basin (Table 1). However, calculated concentrations at those salinities for Mo (31.3–44.5 nM) and Cu (16.1 and 13.6 nM) were higher than those reported for the upper river basin (Mo: 0.5 ± 0.3 nM and 0.4 ± 0.2 nM; Cu: 2.9 ± 0.6 nM and 6.7 ± 4.7 nM, at salinities 5 and 10 respectively). These discrepancies are not totally unexpected for some metals, as inputs from shelf sediments could increase metal levels within the high-salinity end-member of the Amazon plume. For example, Breckel et al. (2005), reported that the Amazon shelf sediment is a significant source of Mo to the ocean (with a estimated flux to the dissolved phase of 0.6×10^7 mol/yr), which could cause an overestimation in our hypothetical low-salinity end-member calculations, due to non-conservative excesses relative to simple mixing of freshwater and seawater. Further studies will need to address all of those issues.

3.2 Trace element composition in *Trichodesmium* colonies

In order to investigate the influence of dissolved metal concentrations on the elemental composition in *Trichodesmium*, we analyzed two different metal pools, intracellular and surface-scavenged, in the colonies collected at the western tropical North Atlantic Ocean (Fig. 1). Total (intracellular + surface-scavenged; Ag, Al, Cd, Co, Cu, Fe, Mn, Mo, Ni, P, Pb, V) and intracellular metal (Cd, Co, Cu, Fe, Mn, Mo, Ni, P, V) concentrations measured in those colonies are reported in Table S1.

Element abundances in the intracellular fraction ranged from 0.49 ± 0.27 mol per colony for P to undetectable levels for Pb (P>Fe>V>Ni>Mo>Zn>Cu>Mn>Co>Cd>Pb) (Table S1). The general concentration pattern seems to be consistent with the metabolic requirements of *Trichodesmium*. For example, P and Fe are well known

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essential nutrients and considered limiting elements for N_2 fixation, oceanic primary production and carbon dioxide sequestration (Sañudo-Wilhelmy et al., 2001; Mills et al., 2004). The high internal content of Fe and Mo in the colonies is consistent with the requirements for these elements by the nitrogenase enzyme for nitrogen fixation (Tuit et al., 2004), and for other Fe proteins involved in aerobic nitrogen fixation in *Trichodesmium* spp. (Zehr et al., 1997).

Explanation for the high internal concentrations of Ni and V are not totally clear.

In the case of Ni, recent research indicates that Ni can be a limiting factor for nitrogen fixation by *Trichodesmium* in oceanic regions (Ho and Hu, 2010). This could be associated with the synthesis of Ni-superoxide dismutase by *Trichodesmium* to eliminate oxygen radicals during the Mehler reaction, as previously shown in other marine cyanobacteria (Dupont et al., 2008). Only two biological roles for V have been identified in cyanobacteria – as cofactors in V-nitrogenases and V-haloperoxidases (VHPOs; Rehder 2008). To date, no marine cyanobacteria are known to possess V-nitrogenases (Walmsley and Kennedy, 1991; Stal and Zehr, 2008). Though VHPO activity has so far only been observed in eukaryotes (Winter and Moore 2009), several cyanobacterial genomes (e.g. *Synechoccus sp* CC931, *Acaryochloris marina* MBIC11017) possess genes both annotated in the IMG database as likely VHPOs and with high sequence homology to characterized eukaryotic VHPOs (http://img.igi.doe.gov). A number of other cyanobacteria, including the published *Trichodesmium* genome, contain an uncharacterized enzyme annotated as VHPO /acid phosphatase-related with predicted peroxidase function. It is possible that the high V quotas measured in this study are due to usage as a cofactor in this uncharacterized enzyme. Vanadate and phosphate are also chemical analogs, which suggest that V incorporation could happen as part of phosphate uptake (Ray et al., 1993).

The relative abundances of the extracellular metal pools (i.e. Cd>Al>V>Ag>Pb>Co >Fe>Mo>Mn>P>Ni>Cu>Zn) follows the sequence characteristic of scavenged elements in the ocean, suggesting that extracellular metal composition is mediated by process of surface-adsorption (Clegg and Sarmiento, 1989).

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In order to provide a comparison of the metal concentrations measured in the colonies independent of cell volume, all of the *Trichodesmium* metal data were normalized to the amount of phosphorous (mol:mol). Averages of intracellular metal: P were 3.5×10^{-5}, 4.7×10^{-5}, 7.4×10^{-4}, 9.9×10^{-4}, 3.2×10^{-3}, 4.5×10^{-3} and 1.5×10^{-2}, for Cd, Co, Mn, Cu, Mo, V, Ni and Fe, respectively (Table S1 and Fig. 3). While these intracellular contents probably denote the biological requirements for these metals, others elements like Mn was mostly found on the cell surface (~74%; Fig. 3), suggestive of a lower cellular demand or lower bioaccumulation rates (Tang and Morel, 2006; Whitfield, 2001).

The range of intracellular metal concentrations measured in the *Trichodesmium* colonies were in agreement with the metal:P range reported for other phytoplankton species (i.e. diatoms *Thalassiosira wissflogii* and *Ethmodiscus rex and gazellae*), grown under laboratory conditions (Table 2), suggesting similar metal requirements among different marine phytoplankton species. The exceptions were Co and V whose concentrations in *Trichodesmium* (Co, range: 0.01–0.14 mmol/mol; V, range: 5.0–11.4 mmol/mol) were one and two order of magnitude higher than in *T. wissflogii* (Co, range: 0.07–0.08 mmol/mol; V, range: 0.018–0.02 mmol/mol, respectively; Table 2). As previously suggested, metal content of field populations of phytoplankton seems to be more variable than those reported in laboratory culture studies (e.g., Tovar-Sanchez et al., 2006).

We found significant linear correlations between the intracellular and the total fraction for many elements measured in the colonies along the spatial gradient of dissolved metal concentrations (Figure 4a–g). The best linear correlations were for Co ($r^2 = 0.69$, $p < 0.001$), Cu ($r^2 = 0.58$, $p < 0.001$), V ($r^2 = 0.56$, $p < 0.001$), Cd ($r^2 = 0.51$, $p = 0.001$) and Mo ($r^2 = 0.37$, $p = 0.008$). Despite the significant correlations between intracellular and total metal pools in the field colonies, prediction of the intracellular pool using the total metal content produced inaccurate results. Those correlations accounted, at the most, for 59% of the measured intracellular Cu (even lower for the rest of the metals; Cd, 17%; Co, 33%; Fe, 21%; Mo, 18%; Ni, 47% and V, 33%). With the exception of

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Cu, for which concentrations in the colonies seems to increase as water column levels increased, in general, trace element concentrations measured in the *Trichodesmium* did not show a clear trend with the dissolved concentrations. These results suggest that the dissolved metal fraction was not a good predictor of the metal trends observed in the colonies (Fig. 4). This is not totally unexpected, as the total dissolved pool includes biologically unavailable metals.

We used a Principal Component Analysis (PCA) to try to indirectly determine whether biological processes such as nitrogen fixation, carbon drawdown and biomass production (measured as chlorophyll *a*) were influenced by trace elements as well as by other physical variables such as salinity, temperature, and mixed layer depth (Fig. 5). All of the biological data (i.e., nitrogen fixation and biological drawdown of DIC) as well as mixed-layer depth measured during our sampling campaign were previously reported by Subramaniam et al. (2008). In our PCA, 69% of total variance was explained by the first two principal components. The first PC is defined by the positive relationship of biomass (as chlorophyll *a*) and the biological drawdown of dissolved inorganic carbon (DIC) with the internal content of Fe, Co, Cd, Cu and Mn, in *Trichodesmium*. The relationship of DIC with Fe and Mn is in agreement with the use of these elements by different enzymes for carbon fixation (Webb et al., 2001; Küper et al., 2008; Tchernov and Lipschultz, 2008). Copper is required by cytochrome oxidase, which has been suggested to be involved in nitrogenase protection in *Trichodesmium spp* (Bergman et al., 1993). Cobalt is required for the synthesis of cobalamin (vitamin B_{12}), as diazotrophs seem to be major producers of this organic growth factor in oligotrophic waters (Bonnet et al., 2010). Although Cd does not seem to be involved in any enzymatic process to fix carbon in *Trichodesmium*, labile forms of Cd in seawater have been found complexed to humic material derived from *Trichodesmium,* suggesting a previous internalization mechanism (Jones et al., 1986).

The PCA also showed that nitrogen fixation during our sampling seems to be influenced by mixed layer depth, instead of intracellular trace elements (PC2). Sañudo-Wilhelmy et al. (2001) also reported that MLD was a major factor controlling nitrogen

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fixation in the subtropical Atlantic. The MLD can increase or decrease primary production by supplying deep nutrients or decreasing the light penetration (Polovina et al., 1995). Most of the dissolved metals also clustered in the PC2 with MLD and salinity, suggesting the influence of the Amazon River and MLD on the dissolved fraction of trace metals. In our area of study, salinity, together with temperature and density, determine the MLD (de Boyer Montégut et al., 2004).

A negative trend was found between nitrogen fixation with dissolved P and the internal content of Mo, Ni and V in *Trichodesmium,* suggesting a biological use of these elements during nitrogen fixation. N_2-fixing diazotrophs may contribute to the low dissolved P concentrations in surface waters in this area, making P the element controlling nitrogen fixation under high-Fe conditions (Sañudo-Wilhelmy et al., 2001). Recently, Ho and Hu (2010) reported that, under low environmental Ni concentrations, growth of *Trichodesmium* may be strongly limited. The minimum intracellular Ni to achieve maximum growth rate has been estimated at about 5.0 mmol to mol of P (Ho and Hu, 2010), which is within the intracellular range quotes obtained in this study (from 0.7 to 11.2 mmol/mol to P; Table S1). On the other hand, while the use of Mo mediated by the enzyme nitrogenase is well known, the biological role of V with the nitrogen metabolism in *Trichodesmium* remains obscure.

In summary, our results show that dissolved trace elements in the Western Tropical North Atlantic are strongly influenced by the Amazon River. The concentrations of dissolved Cd, Co, Ni and V appear to have a conservative behavior relative to simple mixing of freshwater and seawater, while Mo and Cu behave non-conservatively. Analysis of internal and extracellular metal composition in *Trichodesmium* colonies revealed positive correlations between the two pools and differences in abundances. While the extracellular fraction results from a scavenging process, the internal content seems to follow biological requirements of the cyanobacteria. With the exception of Cu, metal content in *Trichodesmium* does not reflect the water column concentration. The PCA analysis indicates that while chlorophyll *a* concentration and the biological drawdown of dissolved inorganic carbon (DIC) by *Trichodesmium* are related to the internal content

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of Fe, Co, Cd, Cu and Mn, nitrogen fixation is limited by the internal concentration of Mo, Ni and V and by dissolved P levels.

Supplementary material related to this article is available online at: http://www.biogeosciences-discuss.net/7/6523/2010/bgd-7-6523-2010-supplement.pdf.

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Table 1. Estimation of metal concentrations in the upper Amazon River Basin. We used the regression equations obtained from metal concentrations measured along a wide salinity range under the influence of the Amazon plume. Values are compared with concentrations reported in the upper Amazon River Basin by Seyler and Boaventura, 2003.

		This work	Amazon Basin (Seyler and Boaventura, 2003)					
	Salinity	Extrapolated concentration	Negro River Nov 99	Negro River May 01	Tapajos River Nov 99	Tapajos River May 01	Juati River May 01	Japura River May 01
Mo (nM)	0	18.1						
	5	31.3					0.2	0.5
	10	44,5	0.1	0.9	0.4	0.6		
Ag (pM)	0	30.5						
	5	27.1						
	10	23.7						
Cd (nM)	0	0.04						
	5	0.10					0.5	1.0
	10	0.16	0.1	2.2	0.1	1.3		
Pb (pM)	0	213.6						
	5	181.9						
	10	150.2						
V (nM)	0	8.7						
	5	11.5					6.8	8.6
	10	14.3	10.6	9.5	6.7	6.9		
Fe (nM)	0	24.8						
	5	21.0						
	10	17.3						
Co (pM)	0	160.9						
	5	145.5					652	1500
	10	130.0	1160	2790	220	670		
Ni (nM)	0	2.6						
	5	2.4					10.3	6.6
	10	2.3	2.6	19.1	nd	11.8		
Cu (nM)	0	18.6						
	5	16.1					3.4	10.0
	10	13.6	3.1	3.1	2.0	3.5		

nd: not determined; Ag, Pb and Fe were not analyzed by Seyler and Boaventura, 2003

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Table 2. Range values of metal concentrations normalized to P *Trichodesmium* and Diatoms.

	[1] *Trichodesmium*	[2] *T. Wissflogii*	[3] *Ethmodiscus*
Cd:P (umol/mol)	9.0–76.5	6.6–8.3	
Co:P (mmol/mol)	0.01–0.14	0.07–0.08	
Cu:P (mmol/mol)	0.19–1.78	0.08–0.09	
Fe:P (mmol/mol)	3.0–44	4.4–43.6	6.5
Mn:P (mmol/mol)	0.02–2.96	3.1–4.1	
Mo:P (mmol/mol)	1.0–10.2		
Ni:P (mmol/mol)	0.7–11.2		
V:P (mmol/mol)	5.0–11.4	0.018–0.02	

[1] This work; [2] Tang and Morel, 2006; [3] Villareal et al., 2007

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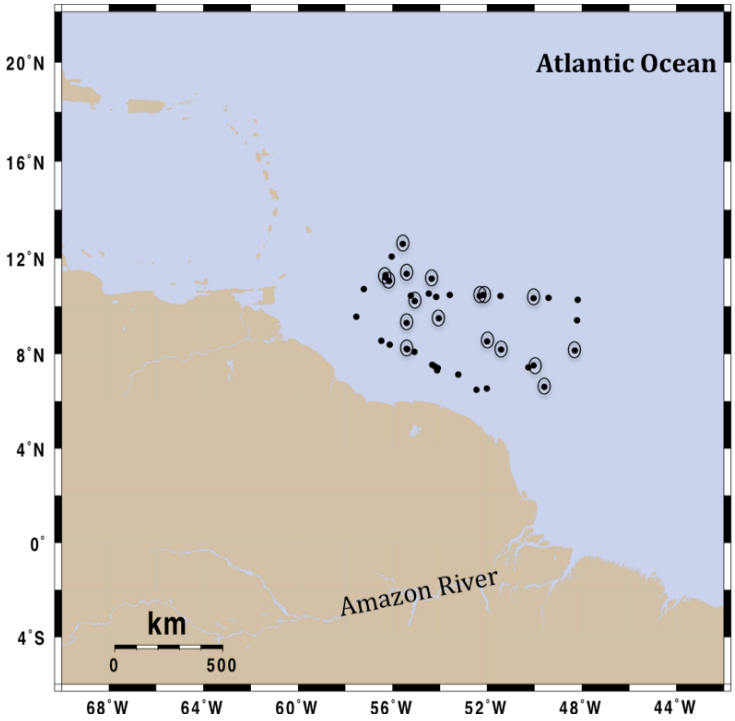

Fig. 1. Sampling locations along the western boundary of the sub-tropical North Atlantic Ocean (filled circles: water samples; open circles: *Trichodesmium* samples).

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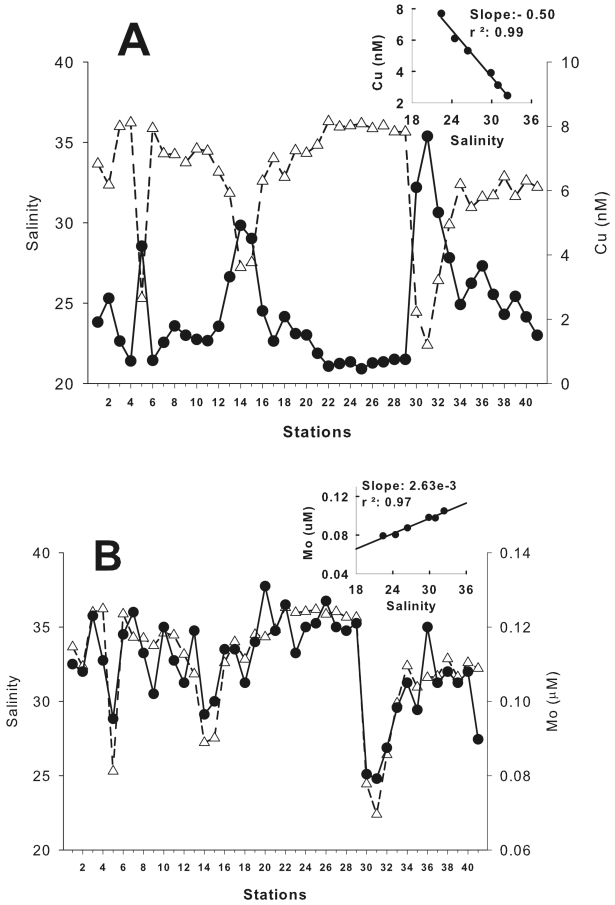

Fig. 2. Geographical distribution of surface salinity (triangles), dissolved Cu (panel A – filled circles) and Mo (panel B – filled circles) measured in the western boundary of the subtropical North Atlantic Ocean. The insets represent linear regressions of the dissolved elements plotted against salinity for six different sampling times over a 24-hour period.

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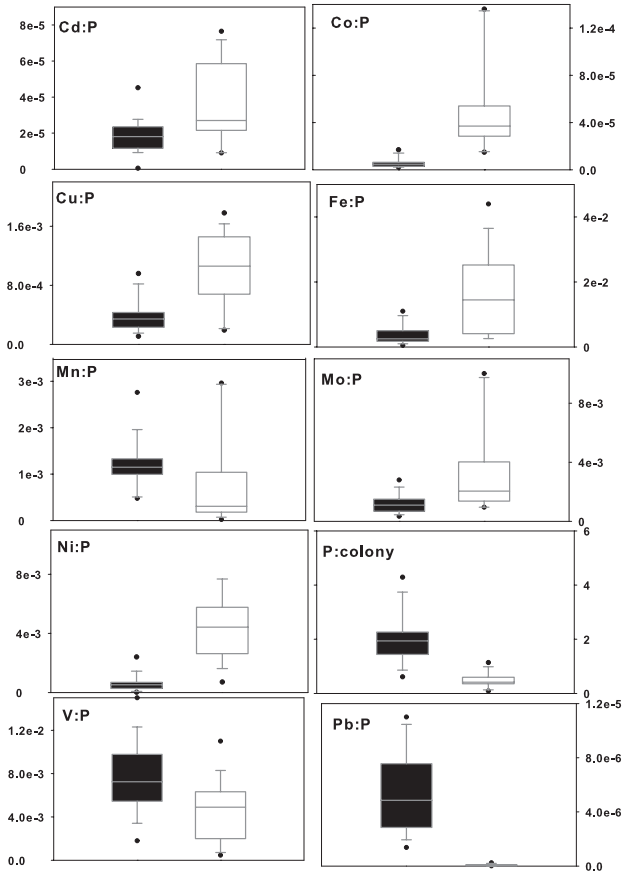

Fig. 3. Box-plots representing the scavenged (filled boxes) and intracellular (open boxes) metal pools measured in field populations of Trichodesmium (all of them in mol:mol, with exception of P that is reported as nmol per colony). The solid line represents the median, the dimensions of the box delineates the 25th and 75th percentile confidence intervals and the error bars show the 10th and 90th percentile confidence intervals.

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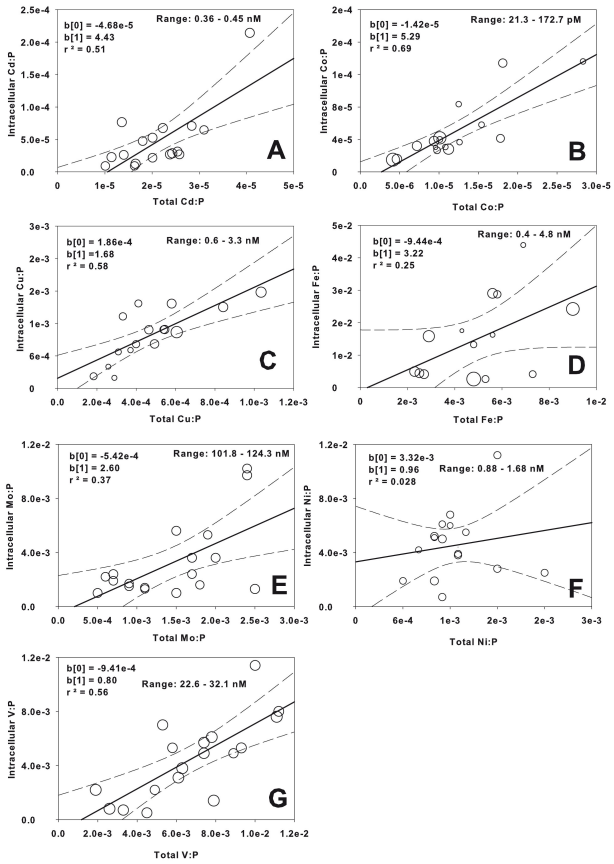

Fig. 4. Bubble plots showing the relationship between total and intracellular metal composition measured in the field colonies of *Trichodesmium* (all metal data normalized to P). The size of the open circles is proportional to the dissolved metal concentrations measured in surface waters where the colonies were collected. The solid line represents the linear regressions obtained among the two biological metal pools and the dashed lines are the 95th confidence limits. The range of dissolved metal levels measured during our study is also shown in each panel.

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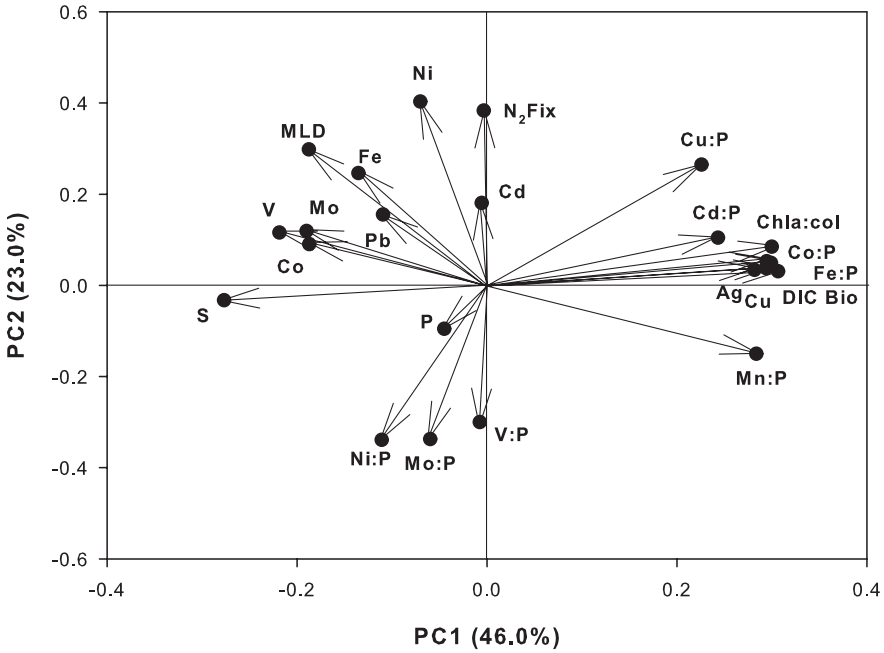

Fig. 5. Principal component analysis for all studied parameters. D issolved elements: Ag, Cd, Co, Cu, Fe, Mo, Ni, P, Pb and V; Salinity: S; Nitrogen fixation: N_2Fix; Mixed Layer Depth: MLD; Biological Drawdown of DIC: DIC Bio; Chlorophyll per colony: Chla:col; Intracellular composition of *Thrichodesmium*: Cd:P, Chla:P, Co:P, Cu:P, Fe:P, Mn:P, Mo:P, Ni:P, and V:P. The first (PC1) and second (PC2) principal components account for 40% and 24.1% of the variance respectively.

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