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Trace Metal Concentration in a Temperate Freshwater Reservoir Seasonally Subjected to Blooms of Toxin-Producing Cyanobacteria

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Abstract In situ interactions between cyanobacteria and metals were studied at Torrão reservoir (Tâmega River, North Portugal). The metal content of water and sediments from the reservoir was monitored monthly at Marco de Canaveses (seasonally subjected to toxic blooms of *Microcystis aeruginosa*) and upstream at Amarante (no blooms recorded), for 16 months. During the 16 months of the study period, *M. aeruginosa* bloomed twice at Marco de Canaveses, firstly forming a scum, and later with colonies scattered throughout the reservoir. Metals Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn were analysed in the sediment and in the water column. Cu-binding ligands in water were also determined. When no blooms were taking place, average metal levels for water and sediment were not statistically different at both locations. Therefore, it was considered that the absence of cyanobacteria blooms at Amarante was not due to differences in metal content. When blooms were taking place at Marco de Canaveses, a significant increase of metal levels in the sediment occurred simultaneously. Sediment quality guidelines showed that during this period, Cu and Pb concentrations (32.3 and 43.2 mg kg^{-1}, respectively) were potentially toxic. However, quantification of the exchangeable metal fraction indicated that these metals were probably not bioavailable. Concentration of Cu-binding ligands in water was higher during the blooms, indicating that cyanobacteria are capable of changing the metal speciation in situ in a reservoir.

Introduction

Cyanobacteria blooms can become potentially hazardous due to the production of cyanotoxins [1]. Moreover, the food web alteration and the generation of hypoxia during harmful algal blooms (HABs) can also represent a threat to the sustainable use of freshwater resources [2]. The occurrence of cyanobacterial blooms has been primarily related to temperature and wind regime [3] as well as nutrient concentration, particularly nitrogen and phosphorus [4], and attempts at modelling the appearance and persistence of blooms, according to these factors, have been made [5]. The contamination of the aquatic systems with inorganic, organo-metallic and organic chemicals has not yet been related to the bloom occurrence, or its decline, but these classes of chemicals are a conspicuous presence in cyanobacteria-inhabited environments.

Requirement and toxicity of metals to cyanobacteria have been reported [6] but seldom in the bloom-forming context. Trace metals elicit a variety of acute and chronic toxicity effects [7], but cyanobacteria also have the capability to accumulate, detoxify or metabolise such contaminants, in a certain concentration range [8]. It is known that cyanobacteria are effective biological metal sorbents, representing an important sink for metals in aquatic environment [9] and that the speciation of many biologically active trace metals, such as Cu or Zn, is partly controlled by metal ligands produced by cyanobacteria [10].

When assessing metals in the aquatic environment, sediments cannot be overlooked, as they work both as a sink and a source. It is known that colonies of the cyanobacterium *Microcystis aeruginosa* may accumulate on the sediments during autumn and winter in a vegetative state [11]. HABs affecting the elemental pool of sediments have been reported, particularly in studies focusing in eutrophic ecosystems, for instance for cyanobacteria [12] and dinoflagellates [13].

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The aims of this work were to measure the concentration and speciation of trace metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in the water and sediments of a temperate freshwater reservoir, seasonally subjected to toxic cyanobacteria blooms, and to elucidate whether there are differences in the water or sediment metal content in locations where cyanobacteria bloom. For this purpose, the Torrão reservoir (integrated in the Douro River watershed that spreads trough the northwest of the Iberian Peninsula) was chosen. This reservoir was created after the building of a dam, in the early 1990s, and has a history of annual seasonal blooms of *M. aeruginosa* Kütz. at Marco de Canaveses [14]; it has a water treatment plant (WTP) but is also affected by a wastewater treatment plant (WWTP) effluent discharge. Since the Torrão dam was built, seasonal blooms dominated by *M. aeruginosa* have occurred at Marco de Canaveses, with microcystin production [15]. Upstream (ca. 20 km) at Amarante, no such blooms have been recorded, but the presence of *M. aeruginosa* toxin-producing strains has been ascertained [15]. To the best of our knowledge, this is one of the few studies dealing with in situ interactions between cyanobacteria and metals.

Materials and Methods

Sampling Area and Sample Collection

Samples were collected at Torrão reservoir, Tâmega River (Portugal—8° 50′ W, 41° 00′ N) at Marco de Canaveses and Amarante. At Marco de Canaveses, the river presents lentic characteristics with an average water depth of 15 m. At Amarante, it has characteristics of a lotic system with an average water depth of 5 m. Sampling took place once a month, for 16 months, from June to October of the following year. The sampling time frame was chosen taking into consideration that blooms of *M. aeruginos*a had been recorded from June to September, in previous years [15].

Sediments from the riverbank were collected in plastic individual bags with a plastic shovel, and water samples were collected in 1.5-L plastic bottles (rinsed and discarded three times before collecting). At Marco de Canaveses, from June to September, water samples were also collected at the place of uptake of the WTP, at three different depths: surface, limit of the photic zone (considered as three times the transparency measured with the Secchi disc) and bottom. Water temperature, dissolved O_2 and pH were measured in situ. Chlorophyll *a* was quantified spectrophotometrically [16], and nitrate and phosphate were determined by photometry (Palintest® Photometer 7000), upon arrival at the laboratory.

Sediment Digestion and Analysis

Sediments were oven-dried at 30 °C and the grain size distribution of sediment was determined in the fraction <2 mm. Grain size analysis was performed by dry sieving (CISA Sieve Shaker Mod. RP.08). Sediments were divided into seven fractions: silt and clay (<0.063 mm), very fine sand (0.063–0.125 mm), fine sand (0.125–0.250 mm), medium sand (0.25–0.50 mm), coarse sand (0.5–1 mm) and very coarse sand (1–2 mm). Each fraction was weighed and expressed as a percentage of the total weight.

HNO_3 digestion of ca. 0.25 g of the sediment fraction <2 mm was performed in a microwave (Milestone MLS-1200 Mega) following the EPA method 3051 [17]. This digestion does not provide total dissolution of the sample, particularly silicates. Nevertheless, it dissolves all elements that could become “environmentally available” [18]. Metal availability was further investigated by sequential extraction (SE), using the method established by the Measuring and Testing Program of the European Community [19]. Only the first step was carried out (ca. 0.5 g sediment was treated with 20 mL of 0.11 mol L^{-1} CH_3COOH), as it is defined as the “exchangeable fraction”.

Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn were determined by atomic absorption spectrophotometry either with flame atomisation (AAS-F) (PU 9200X, Philips) or with electrothermal atomisation (AAS-ET) provided with a Zeeman background correction (4100 ZL, Perkin-Elmer coupled to an AS 70 autosampler), when the levels of metals were too low to be quantified by AAS-F. Aqueous-matched standards were used for external calibrations. To check the accuracy of the analytical procedures, estuarine sediment BCR 277 reference material (certified for total metal content) and BCR 701 (certified for the extractable metal content by SE) were analysed in parallel.

Water Analysis

The water samples were filtered through 0.45-µm nitrocellulose filters (Millipore®). The filters were microwave digested (as described for the sediments), using 1 mL HNO_3, and the metal content was analysed by AAS, as described before. This was considered the particulate fraction.

The 0.45-µm filtered samples were acidified and UV-digested during 1 h, with 1,000-W high-pressure mercury vapour lamp (Ultramed, Osram). Metals were determined by either square wave or differential pulse cathodic (or anodic) stripping voltammetry using an Autolab voltameter (EcoChemie) with a Metrohm 663-VA electrode stand provided with a mercury drop electrode [20].

This was considered the dissolved fraction. Parallel determinations were carried out in the certificate reference seawater CASS-4 (NRC-CNRC).

Determination of Organic Ligands

Labile Cu (Cu_{lab}) concentration, Cu-binding ligand (C_L) concentration, and the conditional stability constant of Cu complexes (K'_{CuL}) were determined by titration of the 0.45-μm filtered sample (not acidified or UV-digested) with Cu^{2+}. Salicylaldoxime (SA) 5 mol L^{-1} was added to tubes already containing Cu in the required range. Solutions were equilibrated overnight at room temperature, prior to titration [20].

Reagents and Material

All chemicals used were of Pro Analysis grade or equivalent (from Merck, Fluka and BDH (Spectrosol)). All material was acid cleaned for 24 h (HCl or HNO_3 20 % (*v*/*v*) for using on voltammetry or AAS, respectively). All metal, ligands and buffer solutions were prepared with bi-deionised water and stored at 4 °C. Bi-deionised water had conductivity <0.1 μS cm^{-1}. All sample manipulations were carried out in a Class 100 laminar flow hood, in a clean room with Class 100 filtered air.

Data Analysis

Metal content results are the mean values of three independent replicates. A one-way analysis of variance (ANOVA) was performed to test for significant differences in mean concentration throughout the sampling months. Whenever significant differences were found ($p \leq 0.05$), a Tukey's test was performed. A *t* test was performed to test for significant differences in mean concentration when data was grouped in blooming or non-blooming periods. Pearson coefficients of correlation (*r*) were employed. Normality of the data was assessed using the Shapiro-Wilk test. STATISTICA 11 (StatSoft®) was used for the calculations.

C_L, K'_{CuL} and Cu concentrations were calculated as described before [20]. The overall side reaction coefficient (α) for Cu was determined using the metal stability constants from Turner et al. [21], and experimental determinations for 0‰ salinity, at room temperature, were performed as described in Campos and van den Berg [22]. Given the fact that all the adopted procedures had a seawater matrix, a comparison test was carried in which ca. 0.5 mol L^{-1} NaCl was added to Torrão freshwater samples. No statistical differences were found ($p \leq 0.05$) for the metal content in samples with or without NaCl addition.

Results

Abiotic Parameters, Chlorophyll *a* and Metal Content at Torrão Reservoir

During the 16 sampling months, two *M. aeruginosa* blooms occurred at Marco de Canaveses. The first took place in October–November, confined to the margin (maximum water depth 0.5 m), where a strip with approximately 1 m wide and 10 m long developed, forming a scum. The second took place in June–July, and colonies of *M. aeruginosa* were scattered throughout the reservoir. The depth of the reservoir was 13 m, and the limit of the photic zone was 5 m. In both blooms, *M. aeruginosa* was identified under an optical microscope [23, 24]. *M. aeruginosa* from Torrão reservoir has been reported as being capable of microcystin production [14, 15], a hepatotoxin, and, therefore, increases of cyanobacterial biomass are considered detrimental for the environment and not suitable for remediation purposes.

During 16 sampling months, average concentrations of dissolved O_2 (9.6±1.3 and 9.0±1.6 mg L^{-1}), nitrate (2.7±0.9 and 3±1 mg L^{-1}) and phosphate (0.11±0.12 and 0.10±0.13 mg L^{-1}), as well as water temperature (16±7 and 18.3±7.2 °C) and pH (7.3±0.9 and 7.1±0.6) at Amarante and Marco de Canaveses, respectively, were not significantly different. Significant positive correlations ($p \leq 0.05$) were found for dissolved O_2, nitrate, phosphate and water temperature, measured during the 16 sampling months in Amarante and Marco de Canaveses. This indicated that the variation across the sampling months followed the same trend in both locations. During periods of cyanobacteria dominance, changes in pH levels have been reported [25], and possibly, this is the reason why pH values were not positively correlated in Marco de Canaveses and Amarante.

Average concentrations of chlorophyll *a* were 7.9±6.7 and 15.3±13.7 μg L^{-1} in Amarante and Marco de Canaveses, respectively. Chlorophyll *a* concentration varied greatly throughout the year, with the highest values from May to September. At Marco de Canaveses, the occurrence of blooms contributed to the higher average level of chlorophyll *a*. Significant positive correlations ($p \leq 0.05$) were found between the phosphate levels and chlorophyll *a* concentration at Marco de Canaveses.

On average, the sediment metal content was higher at Marco de Canaveses than at Amarante for Fe, Mn, Zn, Cu and Pb (Fig. 1). A comparison of the grain size of the sediment at both locations showed that at Marco de Canaveses, the percentage of fine sand plus very fine sand plus silt and clay accounted for 70 % of the <2-mm fraction, whereas at Amarante, the same groups accounted only for 25 % of the total (Fig. 2). This could partly explain the higher metal accumulation in the sediments at Marco de Canaveses. Conversely, on average, the water column metal content was

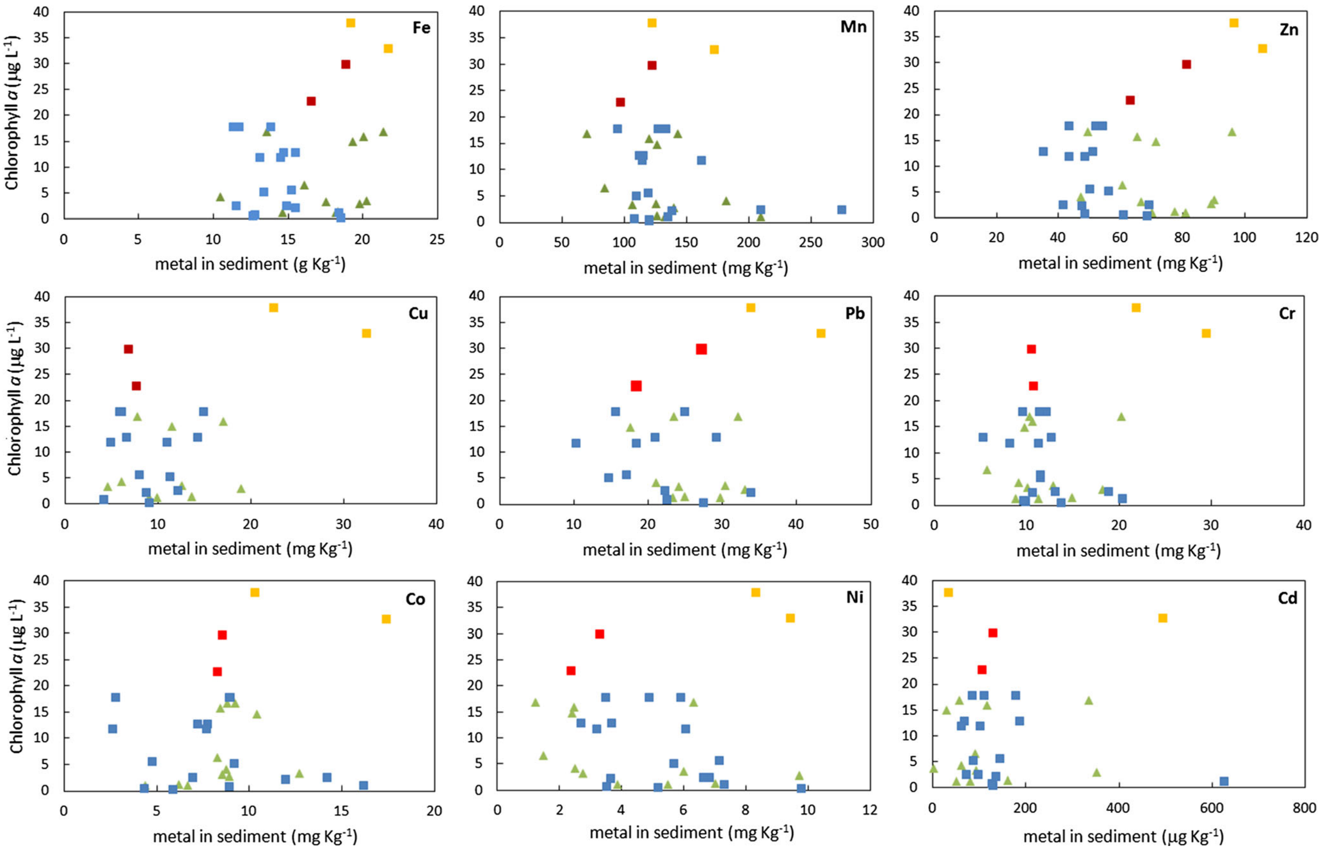
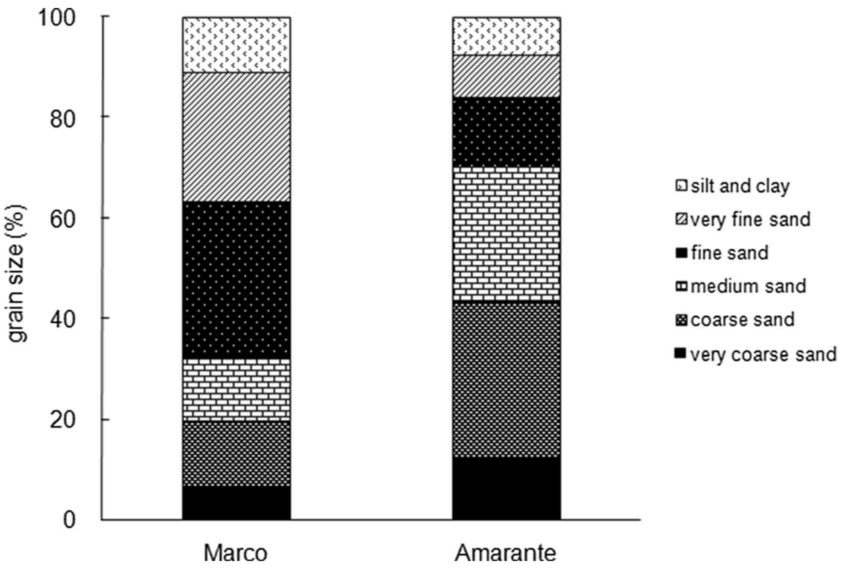


Fig. 1 Metal concentration in the sediment at (*square*) Marco de Canaveses—off blooming in *blue*, scum-forming bloom in *red* and scattered-colonies bloom in *yellow*—and (*triangle*) Amarante in *green*. *Symbols* are the mean value of three replicates; *error bars* are omitted for reading simplicity (colour figure online)

higher at Amarante (Fig. 3). During the occurrence of the blooms at Marco de Canaveses, and particularly the scum-forming bloom, the metal content on the sediment was the highest registered during the 16 sampling months (Fig. 1). For the water metal content, however, Cu and Ni were the only metals to register the highest concentration during the occurrence of blooms (Fig. 3). No correlation could be seen between pH and metal concentrations, at both locations.

Fig. 2 Sediment grain size distribution, in the fraction <2 mm, at Marco de Canaveses and Amarante



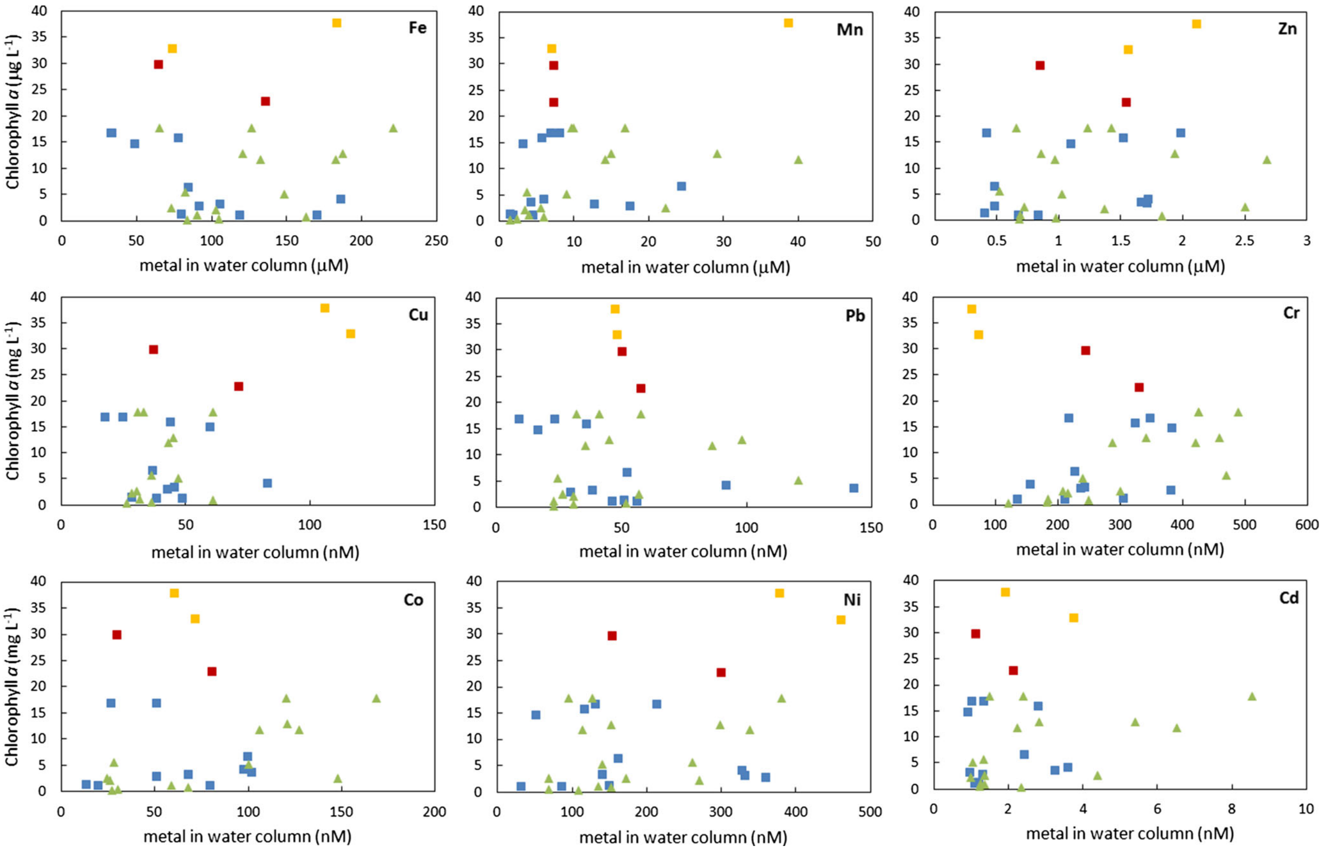


Fig. 3 Metal concentration in the water column at (*square*) Marco de Canaveses—off blooming in *blue*, scum-forming bloom in *red* and scattered-colonies bloom in *yellow*—and (*triangle*) Amarante in *green*. *Symbols* are the mean value of three replicates; *error bars* are omitted for reading simplicity. Results of the particulate and the dissolved metal fraction are shown together (colour figure online)

Metal Content Variation During the Occurrence of a Bloom

The metal levels at Marco de Canaveses were compared during the blooms of *M. aeruginosa* to those occurring during off blooming months. The sediment metal content was higher during blooming, particularly during the scum-forming bloom (Fig. 4a). The analysis of sediment quality guidelines [26] showed that the average metal concentrations were not likely to be harmful, except for Cu and Pb concentrations (32.3 and 43.2 mg kg^{-1}, respectively), which were higher than the threshold effect concentrations (TEC). This prompted the assessment of the metal availability with an SE analysis. The first step of the SE provides the exchangeable metal fraction, which would be the first to reflect any changes in the surrounding environment [21]. The results obtained with the exchangeable metal analysis showed that the sediment metal content was significantly higher during blooming (Fig. 4b), except for Cu and Pb.

In the water column, Cu was the only metal for which the particulate (82.2 nM) and dissolved (30.7 nM) concentrations increased during the blooms. For the remaining metals, the concentrations were not significantly different when comparing the blooming period with the non-blooming period.

When sampling in-depth took place, the pH, dissolved O_2 and water temperature were similar in a blooming and non-blooming situation. Thermal stratification occurred from June to September, but anoxia at the hypolimnion did not occur. The metal levels were similar for the surface and for the limit of the photic zone; however, the particulate and dissolved metal content increased in the bottom. This behaviour was expected, given the physico-chemical characteristics of this reservoir, and the results from other work dealing with vertical metal variation in freshwater reservoirs [27]. Thus, the metal concentration and speciation at the bottom were considered most likely to be controlled by physico-chemical parameters. *M. aeruginosa* is known to possess gas vesicles, which enables the control of the vertical position in the water column, but it mainly occurs in the euphotic zone. Sulphate-reducing bacteria activity was also not expected to occur, as anoxia was not attained.

Cu Speciation

Cu was selected for estimation of the organic ligands concentration owing to its ability in forming the most stable

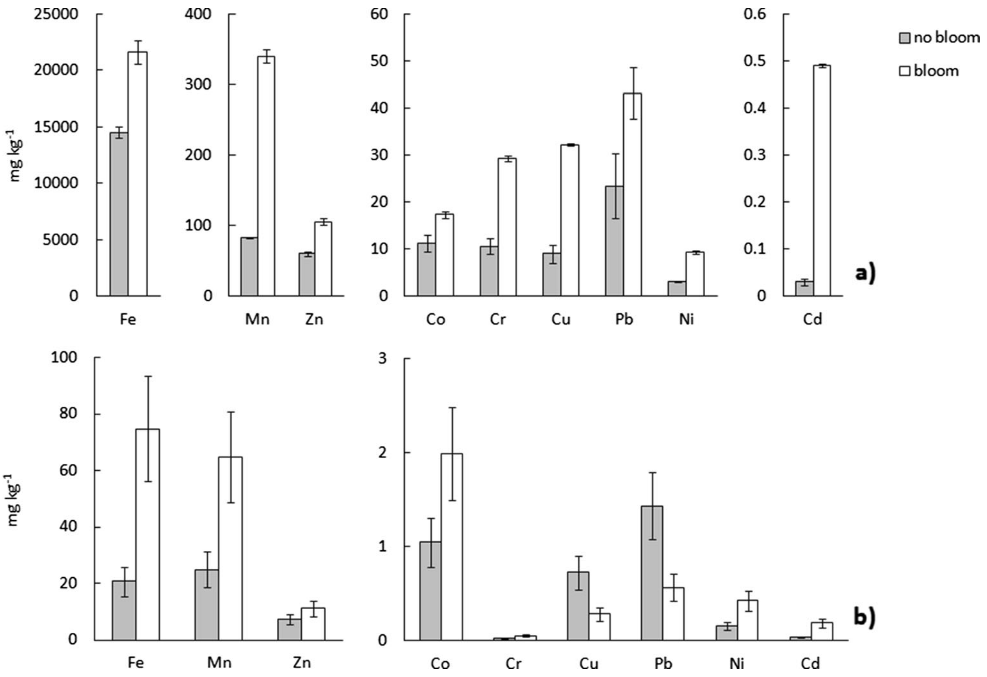


Fig. 4 Environmentally available (digested with HNO_3) metal concentration (**a**) and exchangeable fraction (digested with CH_3COOH) metal concentration (**b**) in the sediment at Marco de Canaveses, in the presence and absence of a scum-forming bloom. Metal concentrations in the bloom are significantly higher ($p \leq 0.05$), except for Cd and Pb in the exchangeable fraction

complexes with the majority of the organic ligands of the Irving-William's series. In fact, Cu was the only metal that showed an increase in concentration in both sediments and water column, during the occurrence of blooms. In the water column, the concentration of Cu increased during the blooms, while the percentage of labile Cu decreased significantly during the blooms (Table 1), being labile Cu the transient chemical specie. The concentration of organic ligands increased significantly during the blooms. However, the conditional stability constant of Cu-binding ligands (K'_{CuL}) was, on average, the same during blooming periods and off blooming periods ($\log K'_{CuL}=12.9$). Therefore, it can be assumed that practically all the dissolved Cu was organically bound at Marco de Canaveses. In spite of that, during blooming periods, the percentage of labile Cu changed, due to the fact that total Cu increased concurrently with Cu-binding ligands.

Table 1 Cu and Cu-binding ligand concentration in surface water of Marco de Canaveses

	$[Cu]_t$ (nM)	$[Cu]_{lab}$ (%)	C_L (nM)
No bloom (*n*=12)	69±26	25±7*	56±15
Bloom (*n*=4)	113±33*	10±3	132±26*

$[Cu]_t$ = particulate + dissolved; $[Cu]_{lab}$ = labile; $[C_L]$ = ligands

Cu labile includes the inorganic Cu that is bound by the added SA (and equilibrates with the Cu added during calibration) and Cu released from organic complexes in competition with the SA added

*significant increase for $p \leq 0.05$

Discussion

In this study, an attempt was made to correlate the appearance of cyanobacteria blooms with environmental characteristics, namely metal content, both in sediments and water column. Other works have detailed the uptake and toxicity of metals in cyanobacteria biomass and in the absence of blooms, as well as during blooms. In this work, the aim was to investigate the metal levels in water and sediments to assess if they could be changed by the presence of cyanobacteria, specifically during blooms. Metals were chosen for being essential micronutrients (Cu, Fe, Mn, Zn) or essential co-factors (Co, Cr, Ni) for cyanobacteria. Cd and Pb do not have known cellular function, but their toxicity has been ascertained and hence their relevance [6].

No differences in sediment or water metal content could account for the occurrence of blooms at Marco de Canaveses and not at Amarante. As *M. aeruginosa* spends part of its life cycle in the sediment, in a vegetative state, the evaluation of the sediment toxicity was particularly pertinent. An analysis of SQGs showed that the average metal concentration, at both locations, was not likely to be harmful [26]. Therefore, the absence of cyanobacteria blooms at Amarante was not likely due to sediment toxicity. Blooms of cyanobacteria are known to be controlled by phosphate levels [28], and in this work, the blooms at Marco de Canaveses coincided with a peak of phosphate. The same increase of phosphate could be seen at Amarante, as the phosphate content was correlated in both locations. At Amarante, there were no blooms, and the lotic characteristics of the river, with more shallow, rapid waters, could be considered the main driving forces for the absence of blooms in this stretch of the Tâmega River.

At Marco de Canaveses, the sediment metal content was found to be higher during the blooming than in off blooming periods. Sediment metal content increase was concurrent with the scum-forming bloom. This bloom took place at a site with maximum water depth of 0.5 m. This would have allowed the cells of *M. aeruginosa* to deposit upon the sediments rapidly, and the presence of cyanobacteria in the sediment interface might have contributed to an increase in metal content. Metal dynamics within the bloom can be substantially different from the ones in the surrounding environment. Other authors reported increases in sediment metal content during the occurrence of a HAB [13] or have shown that during cyanobacteria blooming, the phosphorus content of freshwater sediments changed [12]. The data obtained with the SE showed that an increase in metal content does not imply an increase in available metal content. Cu and Pb higher metal content, detected in the environmentally available extraction, was not reflected in the exchangeable fraction, thought to be the one readily available for the living organisms. It is known that in freshwaters, trace metal distribution is influenced by geochemical and biological processes [29]. But under certain circumstances, bacterial influence may play the dominant role due to high cell density and associated activity [30], contributing to the non-bioavailability of potentially toxic metals, for instance by releasing complexing exudates.

During the blooming, there was an increase in the concentration of organic ligands, capable of changing the bioavailability of Cu. Cu is known to have a preponderant role in freshwater systems [31] and as a micronutrient for cyanobacteria [6], and in fact, Cu was the only metal increasing its content in water (dissolved and particulate) during the occurrence of the blooms. In culture media, excretion of high molecular weight materials (HMWM), capable of efficiently complexing Cu, by *M. aeruginosa* has been established [10]. Algogenic organic matter (AOM) produced by blooms is known to comprise mainly proteins and polysaccharides [32]. Particularly for cultures of *M. aeruginosa*, both at stationary and exponential growth phases, AOM was shown to be dominated (57 %) by hydrophilic compounds [32]. Metals, such as Cu, can bind to these compounds, which are both O and S donors. Despite this evidence in culture media, data concerning this evidence in reservoirs is still scarce. The results of this work show that in situ *M. aeruginosa* exudates are capable of binding metals and change metal speciation in the surrounding media. Thus, blooms of *M. aeruginosa* are capable of changing the metal speciation in temperate freshwater reservoirs and can influence the metal content locally and temporarily, concomitantly with the bloom event.

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