**1**  **The role of biomass elemental composition and ion-exchange in metal sorption**

**2**  **by algae**

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## Abstract

The use of macroalgae, microalgae and cyanobacteria for metal sorption has been widely reported. Still, there are no studies allowing a direct comparison of the performance of these biomasses, especially while evaluating metal competition. The simultaneous sorption of $Co^{2+}$, $Cu^{2+}$, $Ni^{2+}$ and $Zn^{2+}$ present in a multi-elemental solution by six macroalgae, two microalgae and three cyanobacteria was evaluated. Brown macroalgae were shown to be the most promising biosorbent, with *Undaria pinnatifida* having a total metal sorption capacity of 0.6 mmol·$g^{-1}$. Overall, macroalgae performed better than microalgae, followed by cyanobacteria. Carboxyl groups were identified as being the main functional groups involved in metal sorption, and all biomass samples were found to be selective to $Cu^{2+}$. This was linked not only to its higher complexation constant value with relevant functional groups when compared to the remaining metals, but also the Irving-Williams series. The release of $K^+$ and $Ca^{2+}$ to the aqueous solution during the metal sorption was followed. The obtained results suggest they are readily exchanged with metals in the solution, indicating the occurrence of an ion-exchange mechanism in metal sorption by most biomass. Red macroalgae are an exception to the reported trends, suggesting that their metal sorption mechanism may differ from the other biomass types.

**Keywords:** Macroalgae, microalgae, cyanobacteria, screening, sorption mechanism, metal recovery.

## 1. Introduction

The strong dependence of modern societies on metal-rich commodities is putting pressure on natural metal reserves.[1] To avoid short-term supply and demand constraints, the metal industry must pursue a shift from a linear to a circular economy. Wastewaters such as industrial, agricultural and municipal effluents are produced in large volumes and could be viable secondary metal sources.[2–4] Wastewaters have an inherent organic and inorganic heterogeneity which adds complexity to these matrices. Recovering metals from wastewater can be achieved using processes such as hydrometallurgy, electrodeposition, membranes, bioleaching, chemical precipitation and (bio)sorption.[4] Hybrid methodologies can also be developed by combining different processes for better efficiency.[5,6] However, some of these processes are energy and/or solvent-intensive, increasing the overall costs of the process while producing substantial waste volumes.

Biosorption can be a sustainable and cost-effective approach for recovering metals from wastewater.[7] Herein, biosorption is defined as the passive interaction of the biomass surface with metal ions.. Biosorption can occur in living and non-living biomass. The use of non-living biomass for metal sorption has several advantages such as good cost-effectiveness, application in wider pH and metal concentration ranges, no need for additional nutrients and facilitated metal recovery compared to intra-cellular metal accumulation.[8] Non-living biomass can afford quicker and more efficient metal sorption than living biomass[9], also bearing advantages such as control over proliferation and potential fouling events. The success of metal sorption depends on the initial metal concentration, contact time, pH, metal:sorbent ratio and the presence of competing ions.[10,11] Optimization of the sorption process also depends on understanding the underlying mechanisms of metal sorption. Some of the mechanisms involved in metal sorption include physical sorption, chemical sorption and ion-exchange.[12–14] The mechanism(s) involved in metal sorption will also depend on the composition of the used biomass.

Several biomass matrices have been successfully employed for metal sorption, including fruit waste,[15] nuts,[16] spent mushrooms,[11] coffee husk derivatives,[17] and seeds.[18] Macroalgae, microalgae [19,20] and cyanobacteria[21] have also been widely studied for metal sorption, due to their bioavailability, biodegradability, low-cost and efficiency.[9,22] The invasive

57  character of some algae also makes their removal environmentally beneficial. Metal sorption on

58  non-living organisms is related to their cell wall constitution. Metal interaction will mainly occur

59  *via* the functional groups present in the cell wall – carboxyl, hydroxyl, thiol, amino and phosphate

60  groups – and their abundance and availability will determine the success of biosorption.[23]

61  Efforts have been made to find effective biosorbents, with studies comparing the metal sorption

62  efficiency of different macroalgae[20,24,25] and, to a lesser extent, evaluating the efficiency of

63  macroalgae *vs* microalgae[26] and microalgae *vs* cyanobacteria.[27] Still, a simultaneous

64  comparison of macroalgae, microalgae and cyanobacteria under the same experimental

65  conditions seems to be lacking.

66  Algae and cyanobacteria are usually used for water decontamination in general rather than

67  specifically for metal recovery.[28] This causes some divergences in the optimization of metal

68  sorption. Water decontamination seeks the complete removal of metal from the wastewater,

69  often resulting in the addition of high biomass amounts for low metal concentrations.[19,29] In

70  contrast, using biosorption for metal recovery aims at the saturation of the functional groups of

71  the biomass, requiring a good equilibrium between biomass dosage and metal concentration to

72  ensure optimal metal pre-concentration. The selection of target metals and their concentration

73  also depends on the end goal. In water decontamination, it is common to study low metal

74  concentrations, sometimes close to the drinking water and wastewater limits,[30] with target

75  metals being selected due to their toxicity rather than their criticality and economical value.[31]

76  Studies using sorption for metal recovery tend to focus on aqueous solutions with higher metal

77  concentrations and rich in critical or valuable metals, such as acid mine drainage

78  wastewaters.[32] Overall, there is a lack of reports focusing on the use of algae and cyanobacteria

79  as metal pre-concentrators, but also a dearth of studies conducted on multi-elemental metal

80  solutions.[32,33] Most biosorption studies are based on aqueous solutions of a single metal,

81  disregarding the ion competition effect.[19,20,32,33] For metal recovery purposes, it is

82  important to consider metal competition since wastewaters are generally composed of several

83  metals.

84  Herein, a direct comparison of macroalgae, microalgae and cyanobacteria performance is

85  enabled while considering the metal competition effect. Eight algae (six macro- and two

86   microalgae) and three cyanobacteria were screened for metal sorption in multi-elemental

87   aqueous solutions. The metals $Co^{2+}$, $Cu^{2+}$, $Ni^{2+}$ and $Zn^{2+}$ were selected due to their significance for

88   developing low-carbon technology, criticality and their equal valency.[34–36] All biomass

89   samples were characterized through Fourier transform infrared (FTIR), elemental analysis and

90   total reflection X-ray fluorescence spectrometer (TXRF). The correlation between the biomass

91   composition (carbon, hydrogen, nitrogen, sulfur, oxygen, and ash content percents) and sorption

92   capacity was evaluated. The release of $Ca^{2+}$ and $K^+$ ions from the biomass to the aqueous solution

93   was studied to understand the underlying sorption mechanism better.

## 2.   Materials and Methods

95   All chemicals were purchased and used as received. $CoSO_4 \cdot 7H_2O$ (> 99 wt %), $CuSO_4 \cdot 5H_2O$ (>

96   99 wt %) and $ZnSO_4 \cdot 7H_2O$ (> 99 wt %) were obtained from Merck. $NiSO_4 \cdot 6H_2O$ (> 99 wt %) was

97   purchased from Riedel de Haen. Yttrium standard (1000 $mg \cdot L^{-1}$ of Y(III) in 2 wt % nitric acid),

98   poly(vinyl alcohol) (> 99 wt %) and Triton® X-100 (for analysis) were purchased from Sigma

99   Aldrich. All solutions were prepared in ultra-pure water which was obtained through a Millipore

100  filter system MilliQ®. All metal solutions were prepared by gravimetrically weighing (± $10^{-4}$ g) the

101  correct amount of each metal salt. The glass material was previously washed with nitric acid (20

102  v/v %) purchased from Merck (65 wt %) and further rinsed with ultra-pure water.

### 2.1.   Biomass collection and pre-treatment

104  Six macroalgae (*Gracilaria sp.*, *Gelidium sp.*, *Sargassum sp.*, *Saccharina latissima, Ulva rigida*

105  *and Undaria pinnatifida*), two microalgae (*Isochrysis galbana* and *Phaeodactylum tricornutum*)

106  and three cyanobacteria (*Anabaena cylindrica* PCC 7122, *Nostoc muscorum* UTAD_N213 and

107  *Spirulina sp.*) were screened for their metal sorption capacity. *U. rigida*, *Gracilaria sp.*, *U.*

108  *pinnatifida, Sargassum sp. and Gelidium sp.* were kindly provided by ALGAplus, Lda. *Gracilaria*

109  *sp.* was received dry and ground while *U. rigida*, *U. pinnatifida* and *Sargassum sp.* were also

110  received dry, but grinding was performed in the laboratory by freezing the biomass with liquid

111  nitrogen and immediately grinding it with a domestic coffee grinder. The obtained particles were

112  mechanically sieved to select particles with a size under 200 μm. *S. latissima* was kindly provided

113  by Algaia SA (Saint Lô, France). *S. latissima* and *Gelidium sp.* were pre-dried before delivery and

114 ground at lab scale as previously described. *I. galbana* and *P. tricornutum* were acquired dry at

115 Necton S.A. and used without further treatment. *Spirulina sp.* was purchased dry from Shine

116 Superfoods – Alma & Valor and used as received. *A. cylindrica* PCC 7122 and *N. muscorum*

117 UTAD_N213 were cultured in 5 L Schott Duran® glassware containing sterilized liquid Woods Hole

118 culture medium (MBL),[37] in an incubation chamber at $(293 \pm 2)$ K, under a 16:8 h light-dark

119 photoperiod using 2300 lx from cool white fluorescent tubes. After 13 days in culture, the

120 biomass was harvested and concentrated through centrifugation at 277 K and 4111 $g$. The fresh

121 biomass was freeze-dried at < 150 mTorr resorting to a benchtop K, VirTis with a Vacuumbrand

122 pump for one week. All biomass was kept in a dry and light-protected place at room temperature.

123 **2.2.    Biosorption batch studies**

124      Since this work is focused on metal recovery, a fair metal:biomass ratio is required to achieve

125 good metal pre-concentration. In mono-metallic assays with $Cu^{2+}$, $Ni^{2+}$ and $Zn^{2+}$, promising results

126 were achieved with an initial metal concentration of 50 ppm and 500 ppm of non-living algae.[20]

127 Since in multi-elemental assays the metal sorption efficiency is deemed to be lower than in mono-

128 elemental assays, herein the total initial metal concentration was reduced to 40 ppm, with 10

129 ppm each of $Cu^{2+}$, $Co^{2+}$, $Ni^{2+}$ and $Zn^{2+}$ being simultaneously placed in contact with 500 ppm of

130 algae. The pH of the solutions was adjusted to 4 by adding diluted sulfuric acid and determined

131 using a Mettler Toledo SevenMultiTMdual pH meter ($\pm$ 0.02). At low pH values, there is

132 competition between protons and metals for the available binding sites, which limits the metal

133 sorption efficiency of the biomass.[38] In contrast, at high pH values, metal precipitation may

134 occur, once again hindering the metal sorption efficiency. Thus, performing the screening assay

135 at pH = 4 is a reasonable compromise. The batch experiments were conducted in Schott Duran®

136 glassware in an orbital shaker (IKA KS4000 ic control) at 200 rpm and $(303 \pm 1)$ K by adding 500

137 ppm of dried non-living biomass to each multi-elemental metal solution (100 mL). No biomass

138 was added to the control which was simultaneously subjected to the same procedure as the

139 samples. No metal loss was verified in the controls over time. Samples of sorbent suspension

140 were collected (1.5 mL) after 6 and 24 h of contact, centrifuged for 2 min at 12000 rpm and the

141 liquid phase was separated from the residual biomass. All assays were conducted in triplicate.

## 2.3. Metal quantification

Metal quantification was performed by total reflection X-ray fluorescence spectrometry (TXRF) using a Picofox S2 (Bruker Nano (Billerica, MA, USA)) equipped with a molybdenum X-ray source. All the analyses were conducted at a 50 kV voltage and 600 µA current for 300 seconds. Quartz sample carriers were coated with 10 µL of silicon in isopropanol solution and dried at (353 ± 1) K for at least 15 min. A known amount of yttrium was added to each sample and 10 µL of this solution was added to a pre-treated quartz carrier and dried at (353 ± 1) K for at least 30 min.

The amount of metal *per* unit of biomass (sorption capacity, $q$, mmol·g$^{-1}$) at time $t$ was calculated according to Equation 1:

$$q = \frac{V\,(C_0 - C_t)}{m}$$ 
1

where $V$ is the volume of the solution (L), $C_0$ (mmol·L$^{-1}$) is the initial concentration of each metal, $C_t$ (mmol·L$^{-1}$) is the concentration of each metal at that time ($t$) and $m$ is the biomass mass (g). The selectivity of the biomass was determined as shown in Equation 2:

$$S = \frac{q_{metal1}}{q_{metal2}}$$ 
2

where $q_{metal1}$ (mmol·g$^{-1}$) is the sorption capacity of a metal and $q_{metal2}$ (mmol·g$^{-1}$) is the sorption capacity of a second metal.

## 2.4. Biomass characterization

All the evaluated non-living biomass matrices were characterized via Fourier transform infrared (FTIR), elemental analysis and total reflection X-ray fluorescence spectrometry (TXRF). The FTIR spectra of biomass samples were acquired by a PerkinElmer Spectrum BX spectrometer with a diamond crystal and a horizontal Golden Gate attenuated total reflection (ATR) cell. Each sample was analyzed at wavenumbers ranging from 4000 to 400 cm$^{-1}$, with a resolution of 4 cm$^{-1}$ and a total of 32 scans. While the FTIR of the biomass pre-sorption was acquired for all biomass samples the spectra after sorption were only acquired for the macroalgal *Sargassum sp.*, the microalgal *P. tricornutum*) and the cyanobacterium (*Spirulina sp.*). The elemental analysis (C, H, N and S) of the biomass samples was obtained using the equipment LECO TruSpec series 630-

168    200-200 (Michigan, US), whereas the oxygen content was determined by difference after ash

169    content determination. The calcium and potassium content of all biomass matrices was

170    evaluated via TXRF by suspending the biomass in a solution of 0.8 g of poly(vinyl alcohol) (1 wt

171    %) and 0.2 g of Triton® X-100 (1 wt %), spiked with a known concentration of yttrium. The

172    subsequent sample preparation was made as described in sub-section 2.3.

## 3. Results and Discussion

174    The biomass screening included different macroalgae, microalgae and cyanobacteria to

175    facilitate the simultaneous comparison of the metal sorption capacity of different biomass. This

176    is usually done by comparing works conducted under different experimental conditions [20] and

177    limited biomass diversity,[19,39] which hinders the comparison of the sorption capacity of

178    macroalgae, microalgae and cyanobacteria.[19,20,39,40] A comprehensive screening was

179    performed in multi-elemental metal solutions since metal recovery often involves complex

180    matrices. All biomass samples were analyzed by elemental analysis and FTIR to better understand

181    their composition. The presence of inorganic elements in the biomass structure was evaluated

182    by TXRF. The release of ions such as $Ca^{2+}$ and $K^+$ during the sorption process was evaluated to

183    better understand the mechanism behind metal sorption in the different biomass samples.

### 3.1.    Screening assay

185    To evaluate the potential of different algal biomass samples for metal sorption, eleven

186    samples were selected and placed in contact with a multi-elemental solution of $Co^{2+}$, $Cu^{2+}$, $Ni^{2+}$

187    and $Zn^{2+}$, each with a concentration of 10 ppm and at pH = 4. After placing the biomass in contact

188    with the metal solution, samples were collected at 6 and 24 h. No significant differences in the

189    sorption capacity of the biomass were found across time (Figure S1). The sorption capacity of

190    each biomass after 6 h of contact is represented in Figure 1.
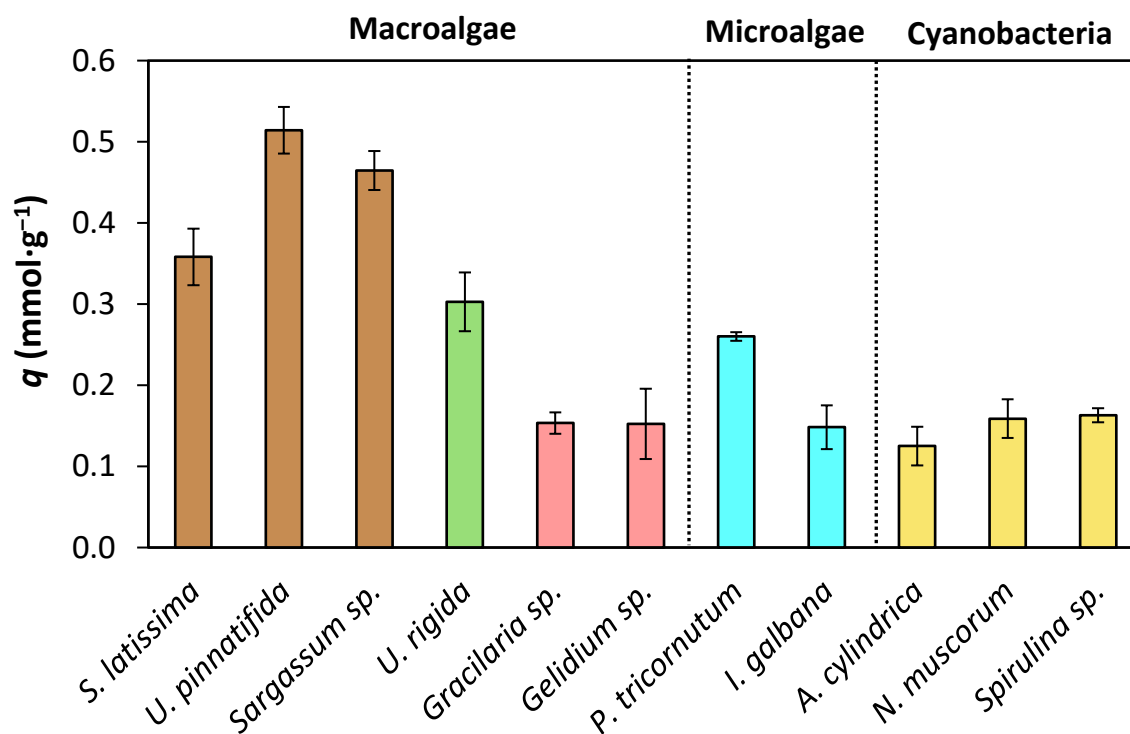
**Figure 1.** Metal sorption capacity ($q$, mmol·g$^{-1}$) of each biomass at a total metal concentration of 40 ppm, 500 ppm of biomass, T = (303 ± 1) K, pH = 4 and t = 6 h. Colors were used to differentiate the biomass samples: brown bars for brown macroalgae, green bars for green macroalgae, pink bars for red macroalgae, blue bars for microalgae and yellow bars for cyanobacteria.

Brown macroalgae afforded better metal sorption capacity values than the remaining biomass types, followed by the green macroalgae *U. rigida.* The red macroalgae showed the lowest sorption capacity within the macroalgae group. Similar tendencies were previously reported in mono-elemental sorption studies, with brown macroalgae being a more promising metal sorbent than green and red macroalgae.[24,25] Brown macroalgae are rich in alginic acid and, therefore, display a large number of carboxyl and hydroxyl groups.[41] This may justify their higher ability for metal sorption, especially at pH values close to the acid dissociation constant of carboxylic acids (pK$_a$ 1.7 – 4.7).[41,42] Sheng *et al*.[43] reported mono-elemental studies using dried non-living *Sargassum sp.*, *Ulva sp.* and *Gracilaria sp.* for the sorption of Cu$^{2+}$, Ni$^{2+}$ and Zn$^{2+}$. Overall, the metal sorption trend was the same as observed here: *Sargassum sp. > Ulva sp. > Gracilaria sp*. The maximum sorption capacity obtained in this mono-elemental assay is significantly higher

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207  than those obtained here. This highlights that the presence of multiple ions in solution promotes

208  intra-system competition and lowers the sorption capacity. Hence, mono-elemental studies do

209  not reflect the real biomass potential for metal sorption in more complex matrices, as

210  wastewaters. As for microalgae, *P. tricornutum* afforded more promising results than *I. galbana*,

211  with its total sorption capacity being similar to that of the green macroalgal *U. rigida.* To the best

212  of our knowledge, there are no reports employing the non-living *P. tricornutum* and *I. galbana*

213  for metal sorption in either mono- or multi-elemental studies. The different sorption capacity of

214  these two microalgae may rely on their structural differences. *P. tricornutum* is a diatom and,

215  therefore, has a silica frustule rich in silanol groups, hydroxyl and carboxyl groups.[44] Although

216  no silanol groups were identified in the FTIR spectrum of *P. tricornutum,* its corresponding peaks

217  may be overlapping with other functional groups. Regarding cyanobacteria, their sorption

218  capacity was comparable to that of the red macroalgae and the microalgae *I. galbana*. Mono-

219  elemental studies involving *Anabaena sp.*, *Nostoc sp.* and *Spirulina platensis*.[45,46] afforded

220  higher sorption capacity values than those obtained herein. Overall, our results reinforce the

221  need to evolve from mono- to multi-elemental studies and enable the establishment of a trend

222  for the biomass sorption capacity that seems to be as follows: cyanobacteria < microalgae <

223  macroalgae.

224      Despite having different metal sorption capacities, all the evaluated biomass samples display

225  a selective metal sorption behavior, as depicted in Figure 2.
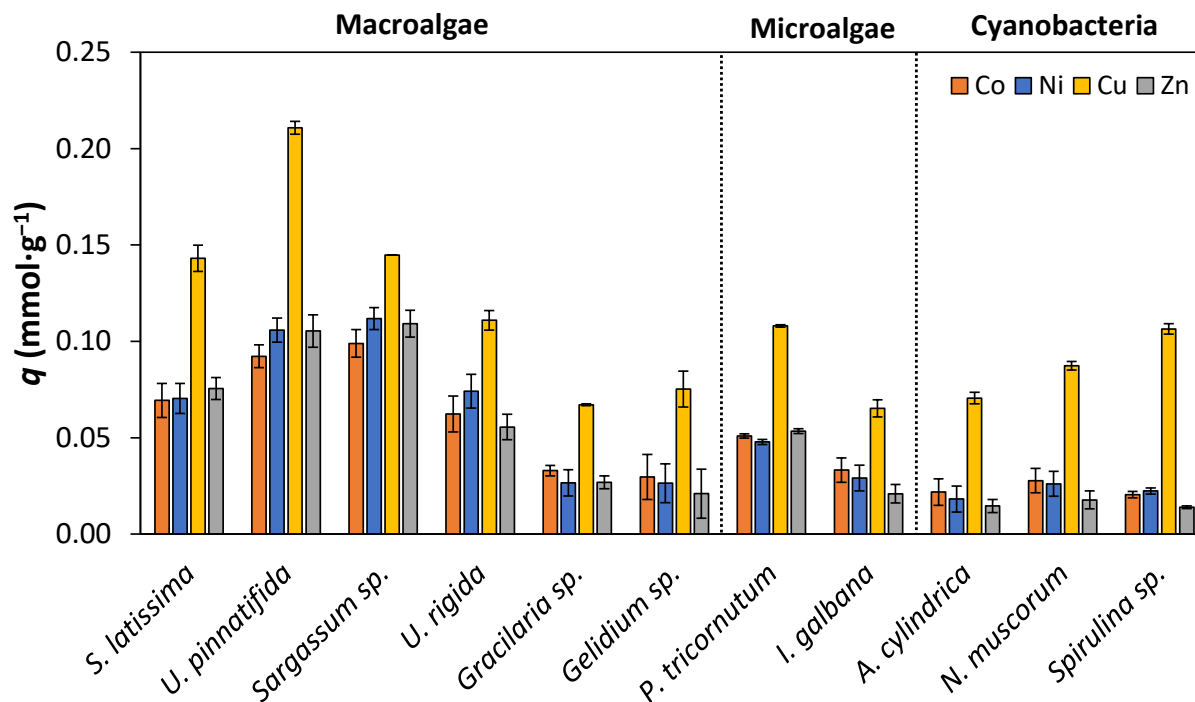
**Figure 2.** Metal sorption capacity ($q$, mmol·g$^{-1}$) of each biomass for Co$^{2+}$ (orange bars), Ni$^{2+}$ (blue bars), Cu$^{2+}$ (yellow bars) and Zn$^{2+}$ (grey bars).

All the studied biomass samples showed a higher affinity for Cu$^{2+}$ than for the remaining metals. No other consistent sorption pattern could be identified across all the biomass samples for Co$^{2+}$, Ni$^{2+}$ and Zn$^{2+}$. Although cyanobacteria were the least effective biosorbent, they displayed the highest relative Cu$^{2+}$ selectivity (see Figure S2). The affinity for Cu$^{2+}$ is likely related to the complexation constant of these metals with the functional groups relevant to sorption. For instance, in the case of a simple carboxylic acid like acetic acid, the logarithm of the complexation constant values for each metal at 298 K and 0 ionic strength is as follows: 1.5 Co$^{2+}$, 2.2 Cu$^{2+}$, 1.4 Ni$^{2+}$ and 1.6 Zn$^{2+}$ (see Table S1).[47] The metal ion Cu$^{2+}$ presents a higher complexation constant value than the remaining evaluated metals and, as a consequence, it is preferentially sorbed. The remaining metals share similar complexation constants, which leads to their indiscriminate sorption onto the biomass. The greater complexation constant value of Cu$^{2+}$, in comparison to Co$^{2+}$, Ni$^{2+}$ and Zn$^{2+}$, is also verified for the hydroxide ion[48] and most amino acids.[49] The preferential removal of Cu$^{2+}$ over Co$^{2+}$, Ni$^{2+}$ and Zn$^{2+}$ was reported in other studies,[20,43] including in multi-elemental sorption assays performed in acid mine drainage wastewaters.[32]

243 The $Cu^{2+}$ selectivity is also in agreement with the Irving-Williams series.[50,51] The Irving-

244 Williams series describes the relative stability order of octahedral complexes formed by $M^{2+}$ first-

245 row transition metal, irrespective of the ligand. The stability order of these complexes for the

246 replacement of water by other ligands is as follows: $Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$.[50,51]

247 These findings are mainly related to the ionic radius of the elements and the crystal field

248 stabilization energy. The higher stability of the octahedral $Cu^{2+}$ complex represents an exception

249 to this due to the Jahn–Teller effect. Briefly, in the case of $Cu^{2+}$, there is an uneven distribution

250 of electrons in the $e_g$ set of orbitals, enabling the possibility to asymmetrically fill the orbitals.

251 This is followed by Jahn–Teller distortion, which causes a tetragonal elongation and the

252 stabilization of the complex.[52] This comparison is facilitated due to the uniform valency of the

253 studied metals ($M^{2+}$). Sorption assays involving metals with different valency will probably impact

254 the trends presented in this work.

255 **3.2.    Biomass characterization and sorption mechanism**

256    The carbon, hydrogen, nitrogen, sulfur, oxygen, ash content (%) and carbon/oxygen ratio (C/O)

257 of the screened biomass is presented in Table 1.

258

**Table 1.** Carbon, hydrogen, nitrogen, sulfur, oxygen, ash content (%) and C/O ratio of the non-living dry biomass.

| | Biomass | % C ± σ | % H ± σ | % N ± σ | % S ± σ | % ash | % O | C/O ratio |
|---|---|---|---|---|---|---|---|---|
| Macroalgae | *S. latissima* | 26.2 ± 0.1 | 4.3 ± 0.1 | 3.94 ± 0.04 | 0.8 ± 0.4 | 36 | 29 | 0.9 |
| | *U. pinnatifida* | 29.1 ± 0.1 | 4.68 ± 0.02 | 2.63 ± 0.03 | 0.8 ± 0.4 | 37 | 25 | 1.1 |
| | *Sargassum sp.* | 37.3 ± 0.2 | 5.0 ± 0.1 | 1.3 ± 0.1 | 0.5 ± 0.1 | 17 | 39 | 1.0 |
| | *U. rigida* | 30.10 ± 0.01 | 5.1 ± 0.1 | 3.78 ± 0.08 | 5.1 ± 0.7 | 32 | 24 | 1.3 |
| | *Gracilaria sp.* | 32.9 ± 0.2 | 4.92 ± 0.08 | 3.41 ± 0.07 | 2.0 ± 0.1 | 27 | 30 | 1.1 |
| | *Gelidium sp.* | 38.3 ± 0.1 | 5.85 ± 0.06 | 3.06 ± 0.07 | 1.9 ± 0.3 | 16 | 35 | 1.1 |
| Microalgae | *P. tricornutum* | 41.3 ± 0.2 | 5.9 ± 0.2 | 6.22 ± 0.04 | 0.8 ± 0.2 | 19 | 26 | 1.6 |
| | *I. galbana* | 48.42 ± 0.03 | 6.63 ± 0.06 | 7.770 ± 0.004 | 0.7 ± 0.1 | 13 | 24 | 2.0 |
| Cyanobacteria | *A. cylindrica* | 45.0 ± 0.2 | 6.4 ± 0.3 | 7.64 ± 0.04 | 0 ± 0 | 4 | 37 | 1.2 |
| | *N. muscorum* | 49.4 ± 0.1 | 6.81 ± 0.01 | 10.97 ± 0.05 | 0.19 ± 0.03 | 3 | 29 | 1.7 |
| | *Spirulina sp.* | 48.9 ± 0.4 | 6.57 ± 0.07 | 11.1 ± 0.3 | 0.55 ± 0.04 | 7 | 25 | 1.9 |

Overall, all biomass samples display high carbon and oxygen content but low hydrogen, nitrogen and sulfur percent. The ash content ranged from 3 to 37 % with macroalgae generally having a higher ash percent than microalgae and cyanobacteria. Macroalgae exhibited low nitrogen content, which could be a reflection of their overall poor protein content, as reported elsewhere.[53] In addition, macroalgae display lower C/O ratio values than microalgae and cyanobacteria. Brown macroalgae (*S. latissima*, *U. pinnatifida* and *Sargassum sp.*) afforded the lowest C/O ratio values, which can be linked to their high alginic acid content and low lipid abundance. [41,53] Both the green (*U. rigida*) and red macroalgae (*Gracilaria sp.* and *Gelidium sp.*) have higher sulfur content than the remaining samples. In the case of the red macroalgae, this is likely related to the abundance of sulfated galactan in their structure.

The biomass elemental composition was plotted against its sorption capacity (*q*, mmol·g$^{-1}$) to study a potential correlation between these parameters (Figure 3). Red macroalgae (*Gracilaria*

273   *sp.* and *Gelidium sp.*) were excluded from this evaluation as they do not follow the trends

274   observed for the other biomass samples.
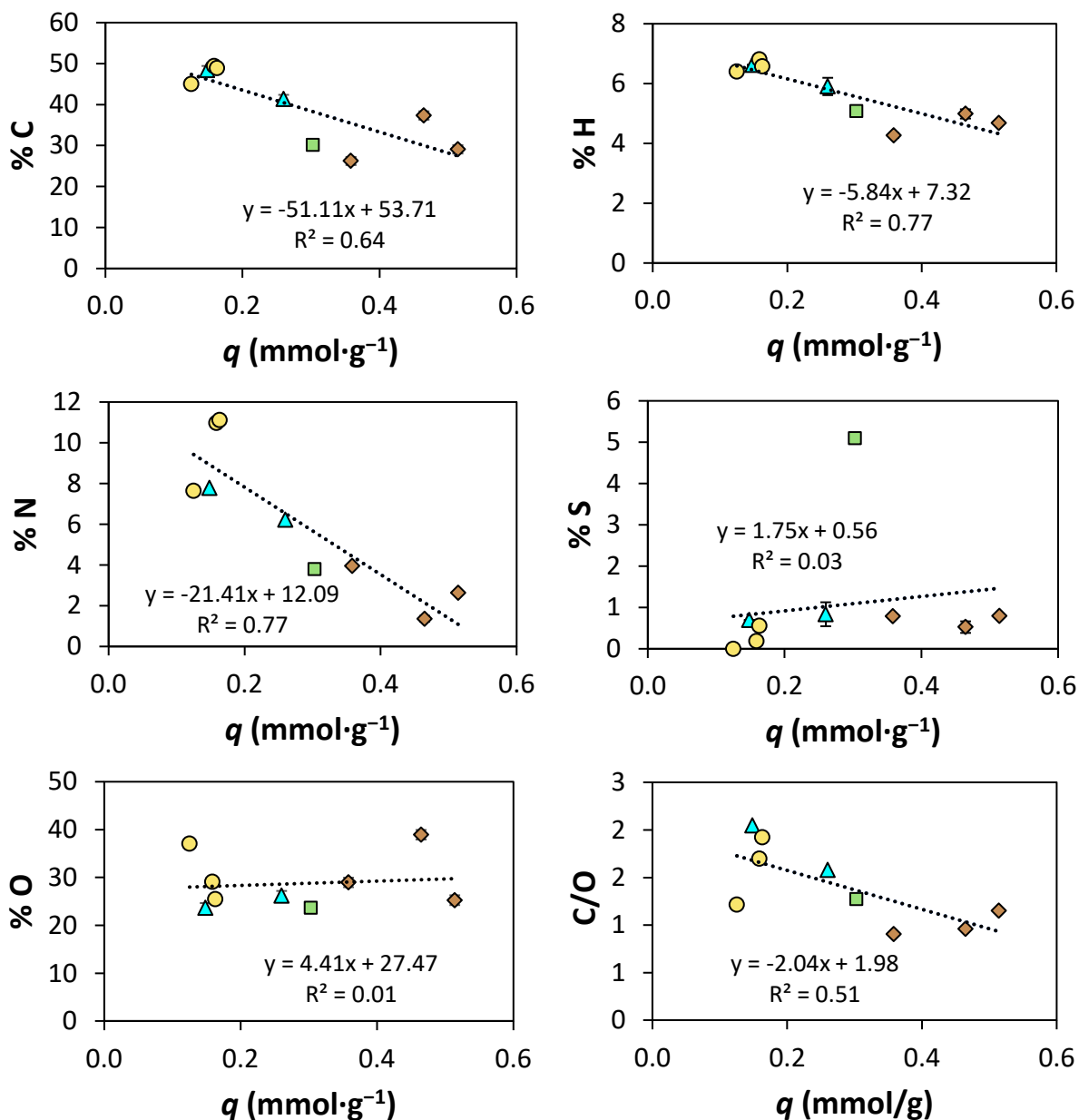


275

276   **Figure 3.** Correlation between carbon, hydrogen, nitrogen, sulfur, oxygen, ash content (%) and

277   carbon/oxygen ratio with the sorption capacity ($q$, mmol·g$^{-1}$) of the screened biomass. Each color

278   represents a set of organisms, brown (◊) corresponding to brown macroalgae, green (□) to green

279   macroalgae, blue (Δ) to microalgae and yellow (○) to cyanobacteria.

280    The data suggest a strong correlation between carbon, nitrogen and hydrogen content and the
281    metal sorption capacity. On the other hand, the sulfur and oxygen biomass content do not seem
282    to be associated with the sorption capacity of the biomass. The plot of C/O ratio *versus* metal
283    sorption capacity seems to suggest that higher C/O ratios are linked to lower sorption capacity
284    values. Still, this dependency is weak and more data would be required to validate this
285    observation. In this work ground non-living biomass was used in all assays. For this reason, there
286    is no distinction between intracellular or surface groups, with all functional groups being available
287    to interact with metal. Establishing these correlations in living biomass is not valid since not all
288    elements will be available for metal interaction, depending on their location.

289    The sorption of metals highly depends on the functional groups present in each biomass
290    sample. FTIR was used to identify the main functional groups of each biomass and details are
291    presented in Tables 2 and 3. Spectra details can be consulted in Figure S3 and S4.

292    **Table 2.** Identification of the main FTIR bands of the studied macroalgae before metal sorption.

| Band origin | Wavenumber ($cm^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | *S. latissima* | *U. pinnatifida* | *Sargassum sp.* | *U. rigida* | *Gracilaria sp.* | *Gelidium sp.* |
| ν O–H (polysaccharides), ν N–H (proteins) | 3269 | 3285 | 3278 | 3208 | 3296 | 3354 |
| ν C–H of aliphatic groups | 2934 | 2925 | 2925 | 2950 | 2924, 2871 | 2927 |
| ν C=O (amide I band) | 1633 | 1622 | 1610 | 1633 | 1644 | 1633 |
| δ N–H (amide II band) | 1538 | 1538 | 1542 | 1548 | 1538 | 1548 |
| δ O–H (carboxyl and hydroxyl groups) | 1416 | 1416 | 1420 | 1404 | 1416 | 1415 |
| δ C–H, δ O–H, (III amide band, proteins) | – | 1241 | 1213 | 1227 | – | – |
| ν C–O (aliphatic ether, primary and secondary alcohol) | 1081, 1021 | 1028 | 1161, 1028 | 1149, 1082 | 1035 | 1149, 1035 |
| ν C–O | 931 | – | – | – | 930 | 931 |

293

294 **Table 3.** Identification of the main FTIR bands of the studied microalgae and cyanobacteria before
295 metal sorption.

| | Wavenumber (cm$^{-1}$) | | | | |
| --- | --- | --- | --- | --- | --- |
| | Microalgae | | Cyanobacteria | | |
| Band origin | *P. tricornutum* | *I. galbana* | *A. cylindrica* | *N. muscorum* | *Spirulina sp.* |
| ν O–H (polysaccharides), ν N–H (proteins) | 3280 | 3274 | 3280 | 3282 | 3280 |
| ν C–H of aliphatic groups | 2959, 2923, 2852 | 2957, 2919, 2849 | 2957, 2926, 2894, 2856 | 2923, 2875, 2852 | 2926, 2874, 2856 |
| ν C=O (amide I band) | 1633 | 1633 | 1644 | 1634 | 1634 |
| δ N–H (amide II band) | 1538 | 1538 | 1538 | 1538 | 1538 |
| δ C–H (methyl and methylene groups) | 1469 | 1454 | 1454 | 1454 | 1454 |
| δ O–H (carboxyl and hydroxyl groups) | 1402 | 1403 | 1393 | 1393 | 1393 |
| δ C–H, δ O–H, (III amide band, proteins) | 1227 | 1234 | 1241 | 1240 | 1239 |
| ν C–O (aliphatic ether, primary and secondary alcohol) | 1039 | 1103, 1072, 1040 | 1151, 1078, 1022 | 1152, 1045 | 1030 |

296

297 According to the FTIR spectra, all the evaluated biomass samples presented O–H and N–H
298 stretching vibrations around 3208 and 3296 cm$^{-1}$, C=O stretching vibration from the amide I band
299 at 1610–1644 cm$^{-1}$ and N–H stretching vibration of the amide II band at 1538–1542 cm$^{-1}$. The
300 FTIR spectra of *Sargassum sp.* showed some changes after contact with the multi-elemental
301 metal solution. For instance, the peak at 1321 cm$^{-1}$ corresponding to the C–O stretching vibration
302 disappeared upon contact with the metal solution. There are also modifications around 1416 cm$^{-1}$
303 $^{1}$, suggesting the involvement of carboxyl groups. In the case of *P. tricornutum*, there are
304 modifications in the amide II band region ($\approx$ 1542 cm$^{-1}$), corresponding to N–H bending
305 vibrations. This macroalgae also presents modifications around 1402 cm$^{-1}$, corresponding to
306 carboxyl and hydroxyl bending vibrations and modifications around 1469 cm$^{-1}$ corresponding to
307 methyl and methylene bending vibrations. *Spirulina sp.* showed no significant alterations before
308 and after metal sorption. Among these three biomass samples, *Spirulina sp.* was the least
309 efficient metal sorbent. The amount of metal sorbed to its cell wall may not be enough to afford

310    visible changes in the FTIR analysis. Of the identified functional groups, the carboxyl groups seem

311    to be significantly involved in metal sorption. At pH = 4 most carboxyl groups are deprotonated

312    ($pK_a$ 1.7 – 4.7) and, therefore, available for metal sorption.[42] Despite the importance of the

313    carboxyl groups for metal sorption, according to the oxygen percent and sorption capacity

314    correlation, the oxygen content alone does not seem to be directly related to the sorption

315    capacity of the biomass, as shown in Figure 1. In this case, the availability of the right type of

316    oxygen-containing functional groups to interact with metals may be more important than their

317    abundance.

318    Since ion-exchange is one of the potential mechanisms behind metal sorption,[54] the

319    inorganic components of the biomass samples were quantified *via* TXRF and are presented in

320    Table S2. The evaluated biomass samples have significant concentrations of $Cl^-$, $K^+$ and $Ca^{2+}$

321    (Figure S5). TXRF is not the most suitable equipment for anion quantification so the presented

322    $Cl^-$ concentration may not be accurate. The abundance of $K^+$ and $Ca^{2+}$ is particularly relevant from

323    an ion-exchange point of view. Other ions such as $Na^+$ and $Mg^{2+}$ are also expected to be in the

324    biomass structure but their quantification is not feasible in TXRF. These ions are generally

325    coordinated with the acidic functional groups of the biomass and can be exchanged by other

326    cations present in the solution. The abundance and diversity of ions in the biomass structure are

327    influenced by the environment where they are grown. For instance, the lab-grown cyanobacteria

328    *A. cylindrica* and *N. muscorum* may display significantly lower amounts of these inorganic ions

329    due to the controlled environment they were grown in. No obvious correlation was found

330    between the $K^+$ and $Ca^{2+}$ concentration on the biomass and its sorption capacity.

331    Considering that, as discussed above, some metal complexes are more stable than others, it

332    is likely that under appropriate conditions a metal can displace another ion from a less stable

333    complex.[50] To better understand the sorption mechanism, besides the $Ca^{2+}$ and $K^+$

334    concentrations present on the biomass samples detailed in Table S2, the $Ca^{2+}$ and $K^+$

335    concentrations were measured for all the controls and samples. While the controls showed no

336    traces of these ions, a significant release of $Ca^{2+}$ and $K^+$ from the biomass to the multi-elemental

337    metal solution was observed in all biomass samples upon metal sorption. The release of these

338    ions was quantified *via* TXRF for all assays and is depicted in Figure S6. The correlation between

339    the release of $Ca^{2+}$ and $K^+$ ions and metal sorption is presented in Figure 4. As in the elemental

340    composition vs $q$ correlation, red macroalgae (*Gracilaria sp.* and *Gelidium sp.*) were excluded

341    from the analyses as they have a behavior clearly different from the other biomass samples

342    studied. The cyanobacterium *P. tricornutum* was also excluded, but only from the $Ca^{2+}$ release vs

343    $q$ correlation, as it significantly impaired the trend ($R^2$ drop from 0.61 to 0.11).

344
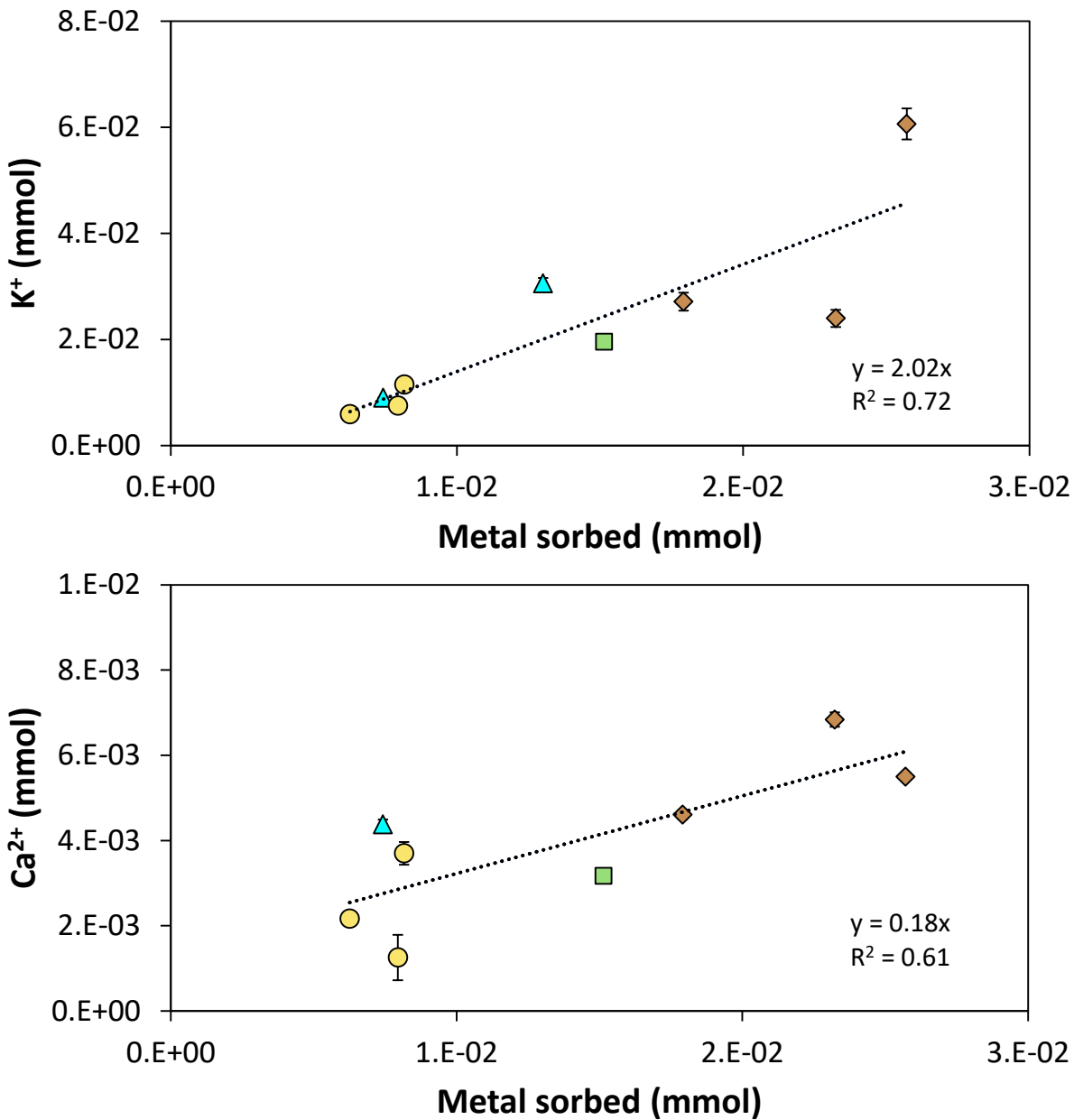


345

346 **Figure 4.** Correlation of the $K^+$ and $Ca^{2+}$ release with the metal sorbed (mmol) for the different

347 biomass samples. Colors were used to differentiate the biomass: brown (◊) for brown

348 macroalgae, green (□) for green macroalgae, blue (Δ) for microalgae and yellow (○) for

349 cyanobacteria. The linear regression is represented by the black dotted line.

350 The $K^+$ release seem to be linked to a large extent to the metal loading into the biomass with 2

351 ions being removed to allow the sorption of a metal ion, as would be expected from the

352 electroneutrality. Although much weaker, the release of $Ca^{2+}$ also seems to contribute to the

353 metal sorption. The total amount of released $K^+$ and $Ca^{2+}$ was charge normalized and compared

354 to the amount of sorbed metals (see Figure S7). Overall, the amount of released ions is very

355 similar to the amount of sorbed metals when considering charge normalization. The only

356 exception is *U. pinnatifida*, where the sum of normalized $K^+$ and $Ca^{2+}$ exceeds the amount of

357 sorbed metals. Since $K^+$ and $Ca^{2+}$ largely account for all sorbed metals, other ions as $Mg^{2+}$ and $Na^+$

358 are unlikely to be involved in the described sorption mechanism. The displacement of these ions

359 confirms the involvement of the ion-exchange mechanism in multi-elemental metal sorption

360 assays. The replacement of the $Ca^{2+}$ and $K^+$ ions can be further related to their complexation

361 constants with the biomass functional groups. According to the complexation constant values of

362 $Ca^{2+}$, $K^+$, $Co^{2+}$, $Cu^{2+}$, $Ni^{2+}$ and $Zn^{2+}$ with the hydroxide ion and carboxylic acids (oxalic and citric

363 acid),[47,48] $Ca^{2+}$ and $K^+$ typically have lower complexation constants than the evaluated

364 transition metals. In the case of the hydroxide ion, the logarithm of the complexation constant

365 values for each metal at 298 K and 0 ionic strength is as follows: 1.3 $Ca^{2+}$, -0.5 $K^+$, 4.3 $Co^{2+}$, 6.3

366 $Cu^{2+}$, 4.1 $Ni^{2+}$ and 5.0 $Zn^{2+}$ (Table S1). When compared to $Ca^{2+}$, $K^+$ has a lower complexation

367 constant. Consequently, $K^+$ should be more easily exchanged with metal ions than $Ca^{2+}$. This is

368 supported by the obtained data since $K^+$ ions were more extensively released from the biomass

369 to the aqueous solution than $Ca^{2+}$ ions (see Figure 4). The red algae always diverge from the

370 presented correlations, suggesting that their metal sorption mechanism may differ from that

371 described for the remaining biomass samples.

372 **4. Conclusions**

373    Eleven non-living algal biomass samples were screened for metal sorption in multi-elemental

374    metal solutions containing equal concentrations of $Co^{2+}$, $Cu^{2+}$, $Ni^{2+}$ and $Zn^{2+}$ at (303 ± 1) K and pH

375    = 4. The composition of the biomass was found to be correlated with their sorption capacity.

376    According to the FTIR spectra of the biomass, carboxyl groups are involved in metal sorption.

377    Despite the strong involvement of oxygen-rich groups in metal sorption, higher biomass oxygen

378    contents do not correlate with better metal sorption capacity values, suggesting that the type,

379    distribution and accessibility of the functional groups are more important than their abundance.

380    Brown macroalgae afforded higher metal sorption capacity values than the remaining evaluated

381    biomass samples. Regardless of being macroalgae, microalgae or cyanobacteria, all biomasses

382    showed a higher affinity for $Cu^{2+}$ sorption. To shed light on the metal sorption mechanism in

383    multi-elemental assays, the release of $Ca^{2+}$ and $K^+$ to the aqueous media was investigated. The

384    obtained results suggest that the release of these ions, in particular $K^+$, is linked to the metal

385    sorption capacity values. This indicates that for most of the studied biomass types, ion-exchange

386    is the prevalent mechanism involved in metal sorption. Altogether, using biomass as a pre-

387    concentrator can be a viable vessel for metal recovery from multi-elemental metal solutions.

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400

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