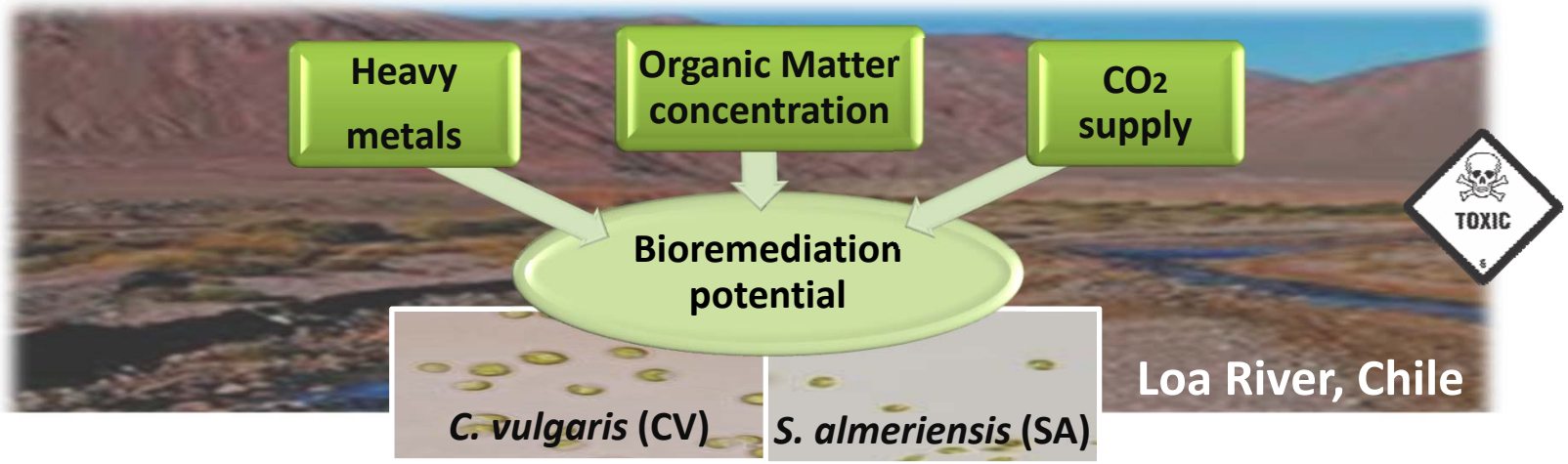
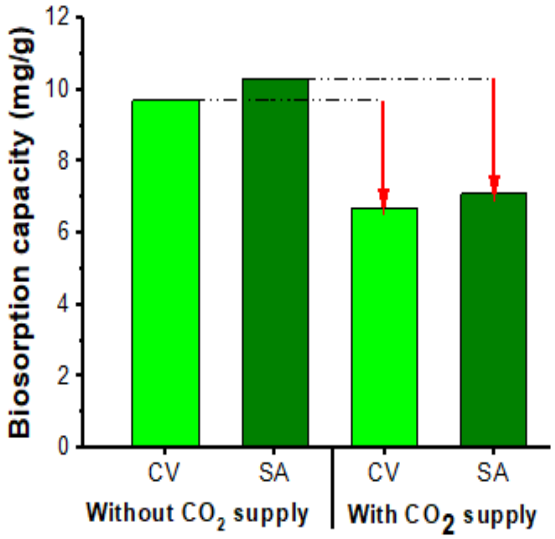
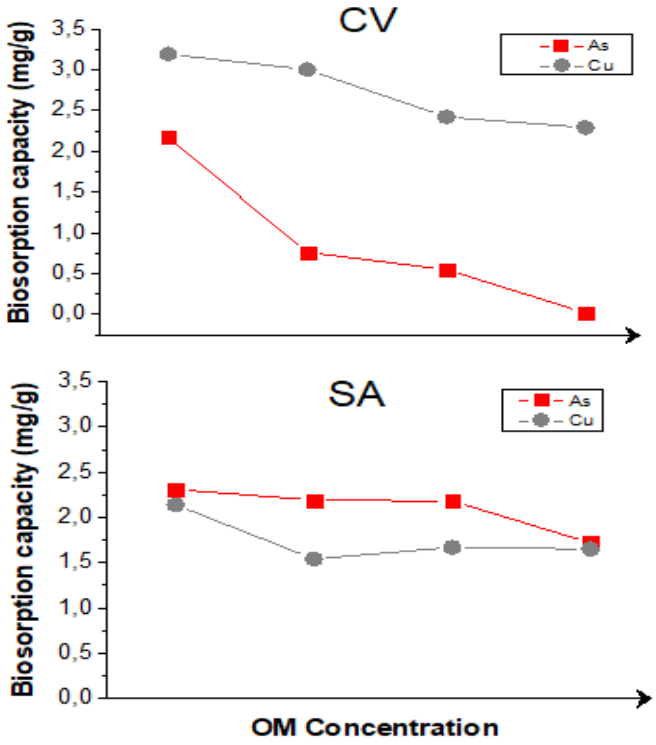
HM Bioremediation by microalgae



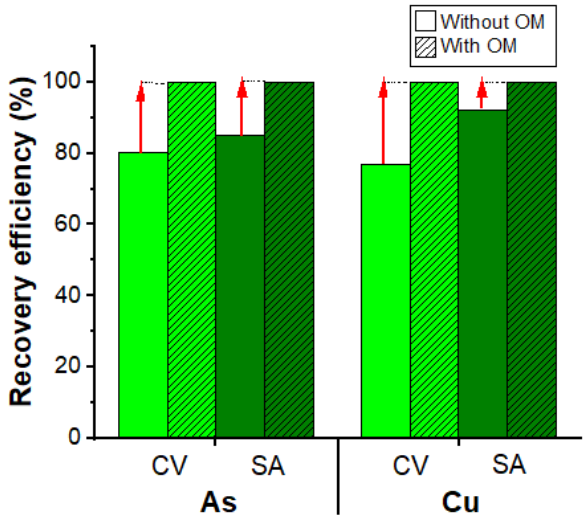
CO_2 supply



Organic Matter Influence



Recovery



HCl 0.1 M

Influence of organic matter and CO_2 supply on bioremediation of heavy metals by *Chlorella vulgaris* and *Scenedesmus almeriensis* in a multimetallic matrix

Ricardo Saavedra[a,b], Raúl Muñoz[a,b], María Elisa Taboada[c], Silvia Bolado[a,b]*

[a] Institute of Sustainable Processes. University of Valladolid, 47011, Calle Dr. Mergelina, s/n, 47011, Valladolid, Spain.

[b] Department of Chemical Engineering and Environmental Technology, University of Valladolid, Calle Dr. Mergelina, s/n, 47011, Valladolid, Spain.

[c] Department of Chemical Engineering, Universidad de Antofagasta, Avenue 02800, CP 1240000, Antofagasta, Chile

*Corresponding author: silvia@iq.uva.es

DECLARATIONS OF INTEREST: NONE

ABSTRACT

This research evaluated the influence of organic matter (OM) and CO_2 addition on the bioremediation potential of two microalgae typically used for wastewater treatment: *Chlorella vulgaris* (CV) and *Scenedesmus almeriensis* (SA). The heavy metal (HM) removal efficiencies and biosorption capacities of both microalgae were determined in multimetallic solutions (As, B, Cu, Mn, and Zn) mimicking the highest pollutant conditions found in the Loa river (Northern Chile). The presence of OM decreased the total biosorption capacity, specially in As (from 2.2 to 0.0 mg/g for CV and from 2.3 to 1.7 mg/g for SA) and Cu (from 3.2 to 2.3 mg/g for CV and from 2.1 to 1.6 mg/g for SA), but its influence declined over time. CO_2 addition decreased the total HM biosorption capacity for both microalgae species and inhibited CV growth. Finally, metal recovery using different eluents (HCl, NaOH, and $CaCl_2$) was evaluated at two different concentrations. HCl 0.1 M provided the highest recovery efficiencies, which supported values over 85% of As, 92% of Cu, and ≈ 100% of Mn and Zn from SA. The presence of OM during the loaded stage resulted in a complete recovery of As, Cu, Mn, and Zn when using HCl 0.1 M as eluent.

Keywords: *bioremediation, CO_2, heavy metal, microalgae, organic matter, toxicity.*

HIGHLIGHTS

- *"SA showed a better bioremediation potential than CV for HM-laden wastewater treatment"*
- *"OM enhanced cell growth but decreased the HM removal 22.7% for CV, and 11.1% for SA."*
- *"CO_2 addition decreased the total biosorption capacity in a 27% for CV, and 35% for SA."*

- *"As was the HM most remarkably affected by the OM presence and CO_2 addition."*
- *"HCl 0.1M resulted more effective than NaOH, $CaCl_2$, and HCl 0.2M for HM recovery."*

1. Introduction

Heavy metal (HM) pollution in bodies of water is an environmental problem that affects millions of people as well as important economic activities worldwide. Conventional technologies such as chemical precipitation, adsorption, ionic exchange, electrochemical treatment and reverse osmosis are expensive, not eco-friendly and not efficient for the treatment of dilute HM laden effluents (<100 mg/L) (Montazer-Rahmati et al., 2011). In addition, the increasingly stringent environmental regulations and public awareness of HM pollution have triggered the quest for novel and cost-competitive remediation technologies.

In this context, biosorption has arisen as a green alternative to conventional technologies for the treatment of effluents containing dilute metal concentrations (Suresh Kumar et al., 2015). This technique is based on the high affinity and fast adsorption kinetics exhibited by organisms such as bacteria, plant, microalgae species, or a mixture of them, towards inorganic pollutants (Bilal et al., 2018; Li et al., 2018; Zhang et al., 2012). In this context, microalgae have emerged as a promising biosorbent for water treatment due to the presence of a high number of functional groups in their cell wall that contribute to the removal of toxic elements from water in a simple, efficient, and economic manner (Zeraatkar et al., 2016). Previous studies have demonstrated the ability of microalgae species to adsorb HM ions from water, which confirms their high bioremediation potential (Cameron et al., 2018; Torres et al., 2017). For instance, a metal biosorption of 22.3 mg Zn (II)/g was obtained in a solution with 150 mg Zn/L, using the green microalgae

Scenedesmus obliquus (Monteiro et al., 2011). Moreover, *C. vulgaris* exhibited a biosorption capacity of 45.4 mg As/g in a solution with 200 mg As/L in an experiment of 7 days of contact time (Jiang et al., 2011).

On the other hand, many recent research efforts have focused on the integration of wastewater treatment (WWT), CO_2 mitigation and bioenergy production processes in microalgae-based photobioreactors (Gani et al., 2017; Rugnini et al., 2019). In this context, domestic and agroindustrial wastewaters contain high concentrations of organic matter (OM) and nutrients suitable for the growth of microalgae (B. Dong et al., 2014; Sahu et al., 2013). The performance of a microalgae-based WWT system depends directly on the capacity of microalgae to assimilate effectively inorganic carbon (CO_2), organic carbon, and nutrients like N and P, with the purpose to produce clean water and low-cost biomass that can be used for biofuels or for the generation of other bio-products (Abou-Shanab et al., 2013; Salama et al., 2017). Nevertheless, typical industrial and livestock wastewaters not only contain nutrients but also hazardous HM. The capacity of the microalgae used in WWT photobioreactors to remove HM in these wastewaters has been widely reported. For instance, a complete reduction of Cu and a 64.96% Zn removal were obtained in *C. vulgaris* cultures in sewage after 10 days at neutral conditions (El-Sheekh et al., 2016). Nevertheless, the biosorption capacity of microalgae in multimetal matrices has been sparsely studied. In this context, the interference of OM and CO_2 supply on HM adsorption has been reported in sediments and soils, but there is a limited understanding of these phenomena in microalgae (Álvarez-Benedí et al., 2005; X. Dong et al., 2014). This work will contribute to elucidate the potential of microalgae biomass for HM removal and the effect of OM concentration and CO_2 supply on the biosorption process ocurring in

72 photobioreactors devoted to WWT. This will provide valuable information about the
73 biosorption mechanisms, applicable also to other microbes.

74 Additionally, HM adsorption on microalgae biomass plays a key role during WWT but
75 can remarkably affect the further valorization processes and bioproduct applications
76 (Bădescu et al., 2018). The recovery of metals improves the economic viability of the
77 process, thereby valorizing the bioremediation process and subsidizing the bioremediation
78 process (Piccini et al., 2019). Therefore, recovery of HM might be needed before biomass
79 processing, which will entail the recovery of HM in a concentrated form (Gupta and
80 Rastogi, 2008). The recovery process can be carried out by proton exchange using eluents
81 such as acid solutions (i.e. HCl, HNO_3), chelating agents (EDTA) or exchange with other
82 ions (i.e. $CaCl_2$) (Vijayaraghavan and Balasubramanian, 2015). Previous studies with
83 *Scenedesmus quadricauda* using HNO_3 0.5 M as the eluent revealed a desorption capacity
84 of 90% for Cd and Pb (Mirghaffari et al., 2014).

85 This research combines the study of the effect of the presence of OM and CO_2 supply
86 on the HM biosorption in a multimetallic solution using microalgae and the evaluation of
87 alternatives of eluents to recover the HM retained in the biomass. Two green microalgae
88 species currently used in studies of photobioreactors for WWT (i.e. *Chlorella vulgaris* and
89 *Scenedesmus almeriensis*) with proven metal adsorption capacity were used as model
90 microorganisms. The toxic elements As, B, Cu, Mn, and Zn were selected as model metals
91 for this study based on the maximum pollution levels recorded in the Loa River basin, the
92 main water source in the Antofagasta region (Chile): an arid region and mining zone with
93 serious problems of water shortage and toxic metal pollution (Romero et al., 2003;
94 Saavedra et al., 2018). Firstly, the effect of the OM concentration on the biosorption of HM

by microalgae was studied, comparing the results with and without CO_2 supply. Variables such as microalgae growth, pH and TOC (total organic carbon) depletion were also monitored for a comprehensive understanding of the OM effect in the bioremediation process. Then, the recovery of toxic elements retained by harvested biomass using three different eluents (HCl, NaOH, $CaCl_2$) at two concentrations (0.1 M and 0.2 M) was evaluated, analyzing metal recovery along time and the effect of OM on the desorption with HCl.

2. Material and methods

2.1. Microalgae cultures and reagents

The microalgae species used in this work were *Chlorella vulgaris* (obtained from the collection of the University of Antofagasta, Chile) and *Scenedesmus almeriensis* (provided by the University of Almeria, Spain). Microalgae biomass was cultivated under axenic conditions using a Bristol medium (UTEX S.A) enriched with a trace metal solution from F/2 of Guillard medium (UTEX S.A) supplying pure CO_2, as reported by (Saavedra et al., 2018). LEDs lamps at 1000 $\mu E/m^2/s$ were used in a 12:12 h:h photoperiod. Cell culture viability and purity were periodically checked by optical microscopy analysis.

Multimetallic solutions of toxic elements were prepared by dissolving a stock solution with 600 mg As /L ($Na_2HAsO_4 \cdot 7H_2O$, Sigma Aldrich), 3000 mg B /L (H_3BO_3, Sigma Aldrich), 150 mg/L of Cu, Mn, and Zn ($CuCl_2 \cdot 2H_2O$, $MnCl_2 \cdot 4H_2O$, and $ZnCl_2$, Sigma Aldrich, Germany) and ultra-pure water under acidic conditions (pH < 3). The stock solution was periodically analyzed and stored at 4°C for quality assurance purposes.

117 Synthetic sewage waters (SSW) were prepared based on typical wastewater
118 compositions by adding (per L of deionized water) 30 mg urea, 28 mg K_2HPO_4, 7 mg
119 NaCl, 4 mg $CaCl_2 \cdot 2H_2O$, and 2 mg $MgSO_4 \cdot 7H_2O$ and varying amounts of peptone and
120 meat extract: C_{OM3} = 160 mg of peptone and 110 mg of meat extract; C_{OM2} = 80 mg of
121 peptone and 55 mg of meat extract; C_{OM1} = 40 mg of peptone and 27.5 mg of meat extract,
122 and C_{OM0} = neither peptone nor meat extract (control test) (Alcántara et al., 2015).

123 NaOH (0.1 M) and HCl (0.1 M) were used for pH adjustment. All the chemicals
124 employed in this study were analytical grade (PANREAC, Barcelona). Plastics and glass
125 containers were immersed in diluted HNO_3 (10% v/v) for 24 h and rinsed three times with
126 Milli-Q water (R > 18MΩ· cm) before use.

127

128 *2.2. Multimetallic biosorption experiments*

129 Biosorption experiments were performed batchwise for both microalgae species at
130 different OM concentrations. The microalgae culture was centrifuged at 4500 rpm for 7
131 min, washed with Milli-Q water to remove the growth medium and centrifuged again prior
132 to the determination of the biomass concentration by gravimetric analysis of the total solid
133 (TS). Synthetic sewage waters of different OM concentrations were added to 4 mL of
134 multimetallic stock solution in order to obtain 200 mL of solution with initial
135 concentrations of toxic elements 12 mg As/L, 60 mg B /L, and 3 mg/L of Cu, Mn, and Zn.
136 Toxic elements and their concentrations were selected according with the composition and
137 maxima concentrations of HM found in the Loa river (Saavedra et al., 2018). The
138 calculated weight of washed and centrifuged biomass were added to these multimetallic
139 solutions to obtain 1 g/L microalgae suspension. The pH value was adjusted to 7.0 in order

140 to simulate the environmental conditions that exist in WWT photobioreactors (Posadas et
141 al., 2015). The tests were conducted in 500 mL glass flasks at 25°C and 1000 μE/m^2/s
142 under a 12:12 h:h photoperiod and magnetic agitation at 250 rpm. Control experiments
143 (without biomass) were conducted at operating pH conditions to check multimetallic
144 solubility. The total contact time was 72 h based on the typical hydraulic residence time
145 used for WWT photobioreactors (Acién et al., 2012). Samples of 8 mL were taken at time
146 0, 3, 24, and 72 hours to determine the pH and HM concentrations. After pH determination,
147 samples were filtered through 0.22 μm membrane filters. Aliquots of 4 ml were acidified
148 with 30μL of HNO_3 (0.1 M) and stored at 4°C prior to metal quantification, while the
149 remaining sample was immediately used for TOC determination. An additional test series
150 was conducted under identical conditions but a constant flow of pure CO_2 was supplied (v
151 > 99.9%, Abello Linde, Spain). The total suspended solid (TSS) concentration (after
152 filtration) in the liquid medium was measured at time 0 and 72 h in order to determine the
153 microalgae growth. TOC removal was determined according to Eq. (1):

154 $$\text{TOC removal } (\%) = (\text{TOC}_0 - \text{TOC}_t)/\text{TOC}_0 \times 100 \quad \text{Eq. (1)}$$

155 where TOC_0 and TOC_t are the TOC concentrations at the initial and sampling times (t),
156 respectively, in (mg/ L). On the other hand, the removal efficiency of each element (i) at
157 time t ($RE_{,i,t}$) was determined by Eq. (2):

158 $$\text{RE}_{i,t} = (\text{C}_{i,0} - \text{C}_{i,t})/\text{C}_{i,0} \times 100 \quad \text{Eq. (2)}$$

159 where $C_{i,o}$ and $C_{i,t}$ are the concentrations at the initial and sampling times (t), respectively,
160 for each toxic element (i) (mg/ L). On the other hand, the biosorption capacity of
161 microalgae for each toxic element (q_{i72}) was calculated by Eq. (3):

162 $$q_{i,72} = \text{V x } (\text{C}_{i,0} - \text{C}_{i,72})/\text{W}_{72} \quad \text{Eq. (3)}$$

where $q_{i,72}$ is the biosorption capacity for each toxic element i: As, B, Cu, Mn, and Zn at 72 h (mg toxic element/g of biomass), V is the volume of suspension (L), $C_{i,0}$ and $C_{i,72}$ are the initial and final toxic elements concentrations (mg/L), respectively, and W_{72} is the mass of dry microalgae at 72 h (g).

2.3. HM Recovery studies

Suspensions of 200 ml containing 1g/L of microalgae, 12 mg/L of As, 60 mg/L of B, and 3 mg/L of Cu, Mn, and Zn were stirred at 250 rpm and 25 ± 1 °C under dark conditions for 3 h in order to load the microalgae with metals. At the end of the contact time, the aqueous phase was removed by centrifugation (4500 rpm, 7 min), and 4 ml of supernatant was filtered through 0.22 µm membrane filters, acidified with 30µL of HNO_3 (0.1 M) and stored at 4° C for the determination of the HM concentrations. Subsequently, 50 mL of the selected eluents (HCl 0.1 M and 0.2 M, NaOH 0.1 M and 0.2 M, $CaCl_2$ 0.1 M and 0.2 M) were added to the centrifuged microalgae. The suspensions were stirred at 250 rpm and 25° C ± 0.1 for 60 minutes. Samples of 4 mL were taken at time 10, 20 and 60 min, filtered through 0.22 µm membrane filters, acidified with 30µL of HNO_3 (0.1 M) and stored at 4°C prior to the analysis of element concentrations. All the experiments were performed in duplicate. The recovery efficiency ($Y_{R,i,t}$) was defined as follows (Eq. (4)):

$$Y_{R,i,t} = (C_{R,i,t} \times V_e)/(W_0 \times q_{1,3}) \times 100 \quad \text{Eq. (4)}$$

where $C_{R,i,t}$ (mg/L) is the concentration of each toxic element (i) in the solution after recovery at sampling time (t), V_e is the volume of eluent (L), W_0 is the initial mass of dry microalgae (g), and $q_{i,3}$ is the initial specific element content of the microalgae (mg/g).

In order to analyze the effect of the presence of OM in the metal loading step on metal recovery, the experiment supporting the highest metal recovery efficiency was repeated under identical operation conditions using C_{OM3} SSW instead of ultrapure water to prepare the multimetallic solution used for the load of toxic elements in the microalgae.

2.4. Analytical procedures

The aqueous concentrations of As, B, Cu, Mn, and Zn were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (HP 7500 cc, Agilent, USA) according to the internal procedures of the Instrumental Techniques Laboratory (LTI – UVa). For quality assurance, two reference water materials were included in the ICP-OES analysis as quality control (QC) samples: ICP multielement Calibration Standard Solution, 100 mg/l Scharlau (26 elements in HNO_3 5%), and a certified Reference Material (Environment Canada TMDA-64.2 LOT 0313, HNO_3: 0.2%) as trace element fortified calibration standard. QC samples were measured every 10 samples, considering a range within 10% of the true value for valid acceptance. Concentrations of dissolved TOC were measured using a Shimadzu TOC-VCSH analyzer (Japan) according to (Marín et al., 2018). The pH was measured using a pH-meter Basic 20+ (Crison, Spain). Determination of TS and TSS concentrations were performed according to standard methods (E.W. Rice, R.B. Baird, A.D. Eaton, 2017).

2.5. Statistical analysis

The influence of TOC concentration, pH, CO_2 supply and ICP-OES analysis was statistically evaluated by correlation and interference analysis (ANOVA). Additionally,

208 results of HM recovery studies were analyzed by one-way ANOVA and t-test ($P< 0.05$).
209 The data were analyzed using the software Minitab 18.
210

211 **3. Results and discussion**

212 *3.1. Effect of OM on the biosorption of toxic elements from the multimetallic solution*
213 *without CO_2 supply*

214 The pH value in *C. vulgaris* cultures remained approximately constant, slightly
215 increasing in solutions with high OM concentrations (values of 7.1 and 7.3 at 72 h in C_{OM2}
216 and C_{OM3}, respectively). These results were consistent with the biomass growth recorded
217 during the experiments: no growth in the control solution C_{OM0} and increases in TSS of
218 2.0% in C_{OM1}, 16.3% in C_{OM2} test and 27.6% in C_{OM3} tests at 72 h. Interestingly, TOC
219 concentrations increased remarkably at 3 h in the biosorption experiments with *C. vulgaris*
220 regardless of the OM concentrations (Fig. 1A). However, the TOC concentration decreased
221 in all the experiments after 24 h of incubation, although positive TOC removals at 72 h
222 were only achieved in C_{OM2} and C_{OM3} test (15.9% and 76.9%, respectively). It is therefore
223 clear that the presence of HM in the medium significantly influenced SSW treatment.

224 On the other hand, the pH in *S. almeriensis* biosorption experiments experienced a
225 remarkable initial increase up to pH 8 at 3 h regardless of the OM concentrations. This fast
226 change in medium pH can be related to the high physiological activity associated to the
227 phototrophic metabolism under this experimental condition (Cabello et al., 2015). From 24
228 h onwards, pH values increased slightly at high OM concentrations (i.e. pH of 8.3 in C_{OM2}
229 at 72 h) and pH decreased in solutions at low OM concentrations (pH of 7.1 in C_{OM0} at 72
230 h). An increase of *S. almeriensis* biomass concentration was recorded in all biosorption

231 experiments, evidencing that the experimental methodology applied did not affect cell
232 viability, and cell growth was correlated with the OM concentration in the solution. Thus,
233 *S. almeriensis* growth at 72 h ranged from 30.6% in C_{OM0} to 40.8% in C_{OM3}. It is known
234 that microalgae support different metabolic processes in order to avoid the high toxicity
235 generated by the presence of HM in the medium. Indeed, extracellular sequestration,
236 intracellular sequestration, active export and enzymatic detoxification rank among the most
237 common processes (Yin et al., 2018). The expression of proteins such as Glutathione S-
238 transferases (GSTs) play an important role in the cell detoxification process, contributing to
239 the protection against oxidative stress generated by the presence of HM (Yin et al., 2016;
240 Zhang et al., 2013). Previous studies showed different growth responses of microalgal
241 species to HM stress. Thus, *Scenedesmus acuminatus* presented a higher antioxidant
242 capacity, lower membrane damage, and higher tolerance than *Chlorella sorokiniana* to
243 stress induced by Cu ions, demonstrating a better tolerance against Cu (Hamed et al., 2017).
244 Therefore, the difference in the pH and cell growth observed between the microalgae
245 studied can be explained by the different metabolic mechanisms used to alleviate HM
246 toxicity.

247 Contrary to the results recorded for *C. vulgaris*, *S. almeriensis* biosorption tests
248 revealed TOC removal from the beginning of the experiment, with a greater removal at
249 higher initial OM concentrations (Fig. 1B). At 3 h, TOC removal was low in all *S.*
250 *almeriensis* biosorption tests, ranging from 0.3% for C_{OM0} to 25.1% for C_{OM3}. From 24 h
251 onwards, TOC removal increased remarkably up to 10.4%, 57.9%, 63.2% and 77.9% at 72
252 h in C_{OM0}, C_{OM1}, C_{OM2}, and C_{OM3} tests, respectively. TOC removal in the medium can be
253 directly associated to the ability of microalgae to use organic carbon as energy source for

mixotrophic growth (Shen et al., 2015). Therefore, the results obtained in *S. almeriensis* tests can be associated to the higher cell growth of this microalga, which supported a superior tolerance over the multimetallic solution compared to *C. vulgaris* during the treatment of wastewater contaminated with metals.

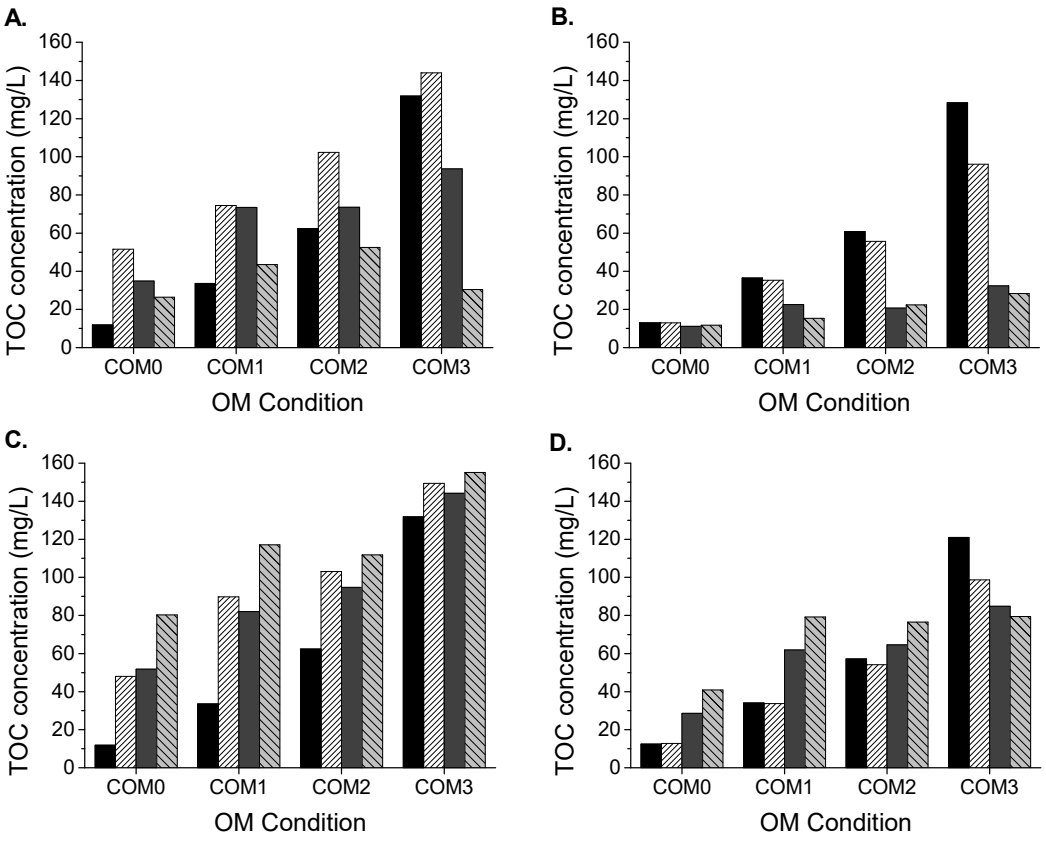


Fig. 1. Time course of the TOC concentration (mg/L) in biosorption studies of *Chlorella vulgaris* (CV) and *Scenedesmus almeriensis* (SA) conducted in a multimetallic solution with different initial OM concentrations at 1 g TSS/L and pH 7.0. A) CV – without CO_2 supply; B) SA – without CO_2 supply; C) CV – with CO_2 supply; D) SA – with CO_2 supply. (■) T_0 (Initial time), (▨) 3 h; (■) 24 h; (▩) 72 h.

265 The influence of OM on the As biosorption in *C. vulgaris* tests was remarkable from the
266 first sampling time at 3 h (with $RE_{As,3}$ 14.2% in C_{OM0} and 3.7% in C_{OM1}). The highest As
267 removal efficiencies were obtained in control tests, where no growth of biomass was
268 observed (Fig. 2A). Interestingly, the As removal efficiencies remained constant from 24 h
269 onwards. The effect of OM was higher in terms of As biosorption capacity of *C. vulgaris*
270 ($q_{As,72}$ of 2.2 mg/g, 0.8 mg/g, 0.5 mg/g, and 0.02 mg/g in C_{OM0}, C_{OM1}, C_{OM2}, and C_{OM3},
271 respectively) than in terms of As removal. Therefore, the presence of OM decreased As
272 removal but promoted biomass growth, thus increasing biomass concentration.

273 Overall, the As removal efficiencies for *S. almeriensis* were higher than for *C. vulgaris*
274 (Fig. 2B). The highest As removal efficiency of *S. almeriensis* was obtained in the control
275 solution C_{OM0} at 3 h (32.4%), which decreased with the contact time to ~ 20% by 72 h,
276 similar to the observations in the other SSW tested. Differences in the final calculated $q_{As,72}$
277 in *S. almeriensis* tests were inversely correlated with cell growth, descending from 2.3 mg/g
278 in C_{OM0} to 1.7 mg/g in C_{OM3}. The lower influence of OM on the As biosorption capacity of
279 *S. almeriensis* compared to *C. vulgaris* could be related to its higher TOC removal capacity,
280 which supported similar final OM concentrations in all the experiments.

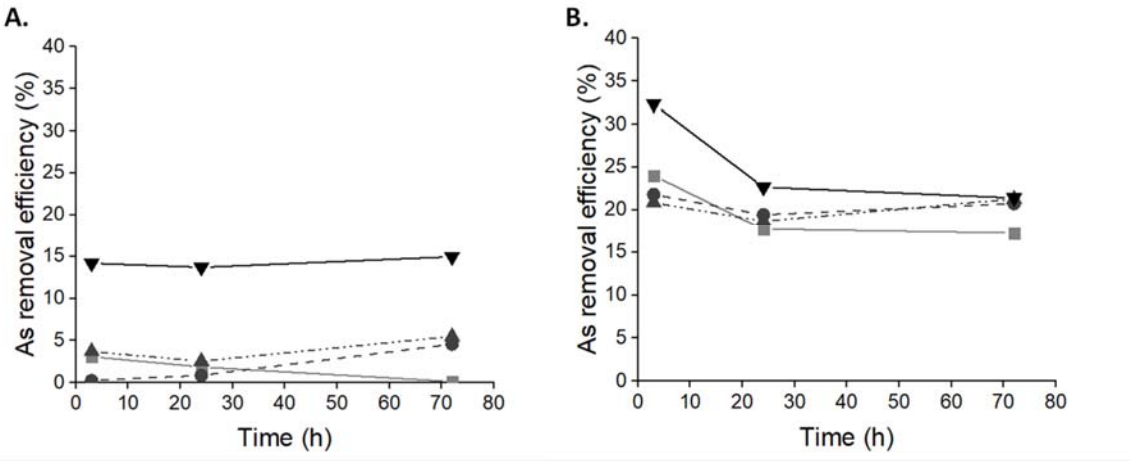


Fig. 2. Time course of the removal efficiency (%) of As in biosorption studies without CO_2 supply at different initial OM concentrations by *Chlorella vulgaris* (A), and *Scenedesmus almeriensis* (B) cultures at 1g TSS/L and pH 7.0. (—▼—): C_{OM0} (control); (- · ▲ · -): C_{OM1} (40 mg peptone /L and 27.5 mg meat extract /L); (- ● -): C_{OM2} (80 mg peptone /L and 55 mg meat extract /L), and (—■—): C_{OM3} (160 mg peptone /L and 110 mg meat extract /L).

The As biosorption capacities obtained in the control tests in this study were lower than those previously reported by Saavedra et al, (2018) in multimetallic solutions at the same metal concentrations, but without the addition of urea and mineral salts. It has been hypothesized that some of the added compounds such as urea or phosphates, besides enhancing the growth and resistance of *S. almeriensis*, could compete with As for the adsorption sites, decreasing the As uptake by microalgae cells (Yamani et al., 2016).

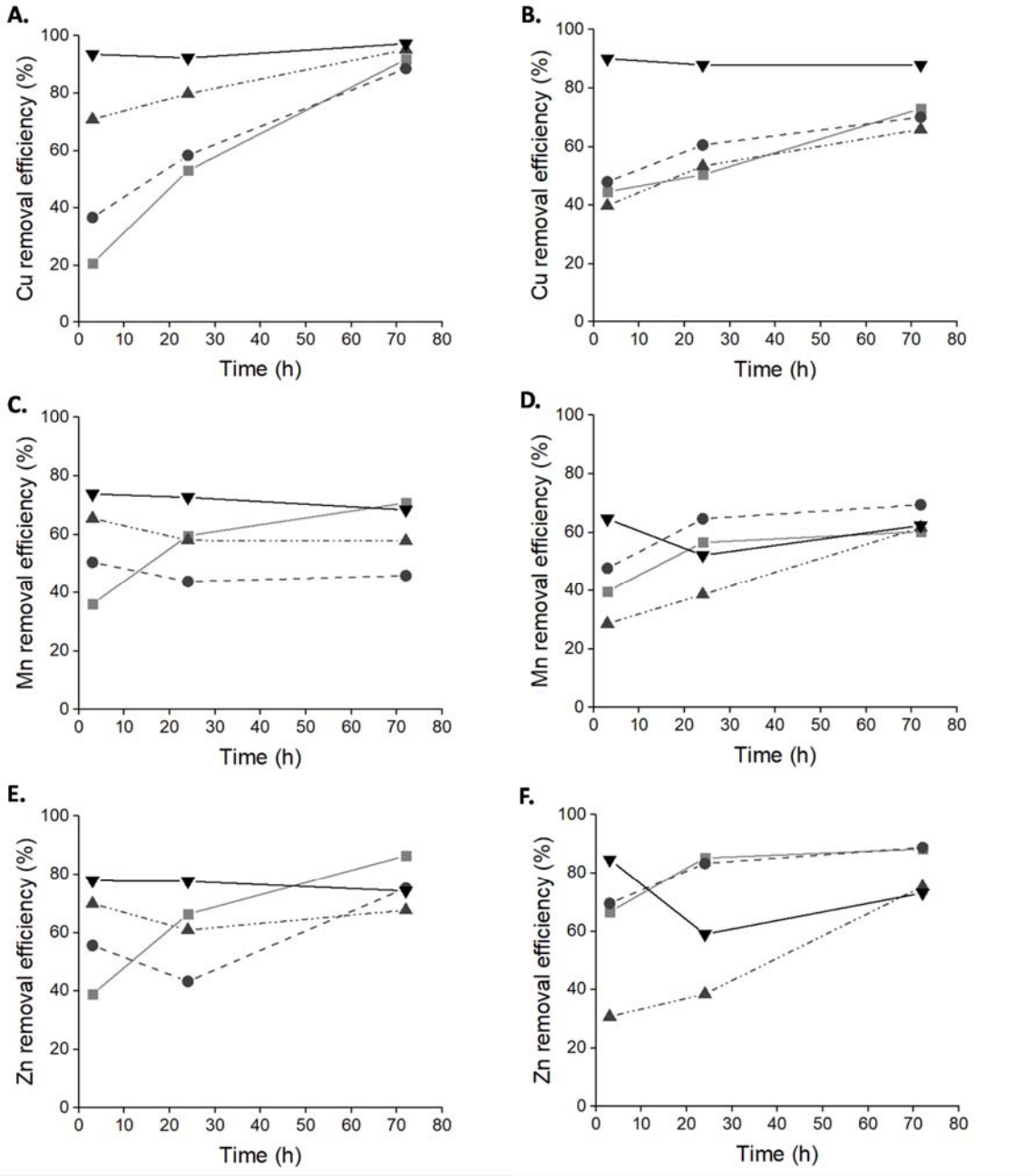
B removal efficiencies were low and relatively stable over time, with a maximum of 8.6% in $RE_{B,24}$ for *S. almeriensis* at C_{OM1} (Supplementary material). The B biosorption capacity of *C. vulgaris* increased with the OM concentration of the SSW (1.4 mg/g, 2.1 mg/g, 2.3 mg/g and 2.6 mg/g in C_{OM0}, C_{OM1}, C_{OM2}, and C_{OM3}, respectively). These results agree with previous studies that reported an uptake capacity of 2.7 mg B/g for *Chlorella* sp.

298 at pH 7.0 (Taştan et al., 2012). Nevertheless, no clear influence of the initial OM
299 concentration was observed in B biosorption capacities of *S. almeriensis,* with $q_{B,72}$ values
300 ranging from 3.2 mg/g in C_{OM0} to 3.7 mg/g in $C_{OM2.}$ The biosorption capacities for B in the
301 control tests were higher than those previously found in the multimetallic solution without
302 the addition of urea and minerals (Saavedra et al., 2018), but these differences were lower
303 for the overall bioremediation process.

304 *C. vulgaris* showed an inverse correlation between RE for Cu, Mn, and Zn and the
305 initial OM concentration at the first sampling time (Fig. 3A, C, and D). For instance, the
306 $RE_{Cu,3}$ accounted for 93.6%, 70.9%, 36.7% and 20.7% in C_{OM0}, C_{OM1}, $C_{OM2,}$ and C_{OM3},
307 respectively. Nevertheless, this inverse correlation between OM and RE was not
308 maintained over time. In *C. vulgaris*, the RE_{Cu} increased with the contact time, especially in
309 the C_{OM2} and C_{OM3} tests ($RE_{Cu,72}$ > 88.6%), although $q_{Cu,72}$ decreased from 3.2 mg/g in C_{OM0}
310 to 2.3 mg/g in C_{OM3}. Mn and Zn removal efficiencies in *C. vulgaris* also increased with
311 time in C_{OM3} solutions, achieving values of 70.8% and 86.4% at 72 h, respectively. The
312 increase observed in Cu, Mn, and Zn removal efficiencies by *C. vulgaris* in high OM
313 concentration tests was likely due to the gradual decrease in TOC concentration (Hussain et
314 al., 2017), but also to cellular growth, which enhanced biosorption by increasing the
315 adsorption sites. Finally, *C. vulgaris* growth likely mediated differences in the Mn and Zn
316 biosorption capacities, with values of $q_{Mn,72}$ of 2.4 mg/g in C_{OM0} and 1.5 mg/g in C_{OM2}, and
317 $q_{Zn,72}$ values ranging from 2.7 mg/g in C_{OM0} to 2.3 mg/g in $C_{OM2.}$

318 Remarkable differences in the RE of Cu, Mn, and Zn in *S. almeriensis* biosorption tests
319 were recorded as a function of the initial OM conditions during the first 3 h (Fig. 3B, D,
320 and F). The highest RE_{Cu} was measured in the control tests, with values almost independent

of contact time ($RE_{Cu,3}$ of 90.1% and $RE_{Cu,72}$ of 88%). Similar RE_{Cu} values in C_{OM1}, $CO_{M2,}$ and C_{OM3} were reported in *S. almeriensis* cultures, which increased over time from 39.8% at 3 h in C_{OM1} to 73% at 72 h in C_{OM3}. Similarly, Cu biosorption capacities of this microalga reached a maximum $q_{Cu,72}$ of 2.1 mg/g in the control test, which was higher than the $q_{Cu,72}$ of 1.5 mg/g, 1.7 mg/g and 1.6 mg/g determined in C_{OM1}, C_{OM2}, $C_{OM3,}$ respectively. These lower values on RE_{Cu} demostrated that the OM effect produced a decrease on Cu removal, occuring independently of the the initial OM concentration in the SSW with added peptone and meat extract.



329

330 **Fig. 3**. Time course of the removal efficiency (%) of copper (Cu), manganese (Mn), and

331 zinc (Zn); in biosorption studies without CO_2 supply at different initial OM concentrations

332 by *Chlorella vulgaris* (CV) and by *Scenedesmus almeriensis* (SA) cultures at 1g TSS/L and

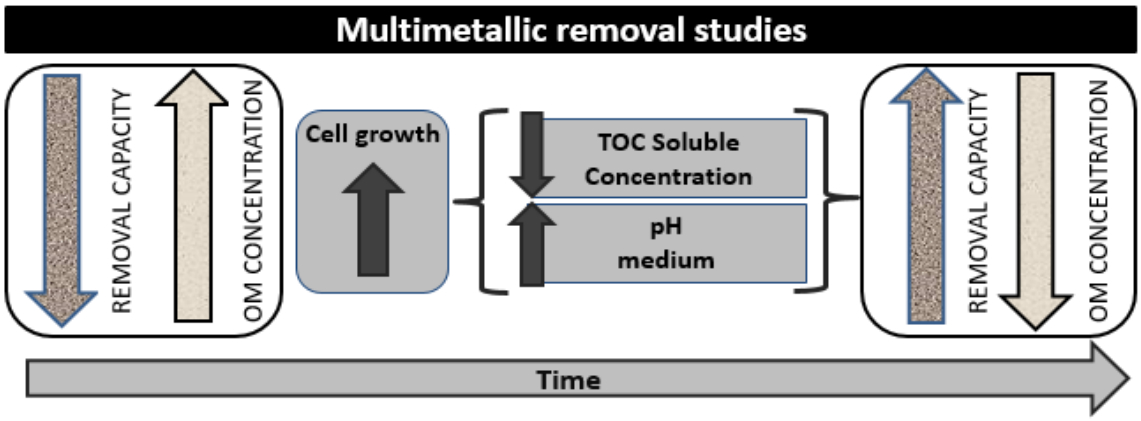
333 pH 7.0. A) CV-Cu; B) SA-Cu; C) CV-Mn; D) SA-Mn; E) CV- Zn; F) SA-Zn. (—▼—): C_{OM0}

334 (control); (- - ▲ - -): C_{OM1} (40 mg peptone /L and 27.5 mg meat extract /L); (– ● –): C_{OM2} (80

335 mg peptone /L and 55 mg meat extract /L), and (—▪—): C_{OM3} (160 mg peptone /L and 110
336 mg meat extract /L).
337
338 Although RE_{Mn} and RE_{Zn} increased in the tests conducted with time in SSW with added
339 peptone and meat extract, the removal efficiency of Mn and Zn by *S. almeriensis* decreased
340 with the contact time in the control tests. The lowest Mn and Zn removal efficiencies were
341 obtained at 3 h in C_{OM1} (28.6% and 30.7%, respectively). The Mn removal efficiencies and
342 biosorption capacities at 72 h reached similar values, independently of the initial OM
343 concentration (≈ 63% and 1.6 mg/g). Larger differences were obtained in the Zn
344 biosorption test using *S. almeriensis*: $RE_{,Zn,72}$ of ~ 74% and $q_{Zn,72}$ of ~ 2.0 mg/g in C_{OM0} and
345 C_{OM1}, and $RE_{,Zn,72}$ of ~ 88% and $q_{Zn,72}$ of 2.4 mg/g in C_{OM2} and C_{OM3}.

346 The biosorption capacities based on the total mass of elements adsorbed at 72 h revealed
347 similar values for both microalgae studied. Interestingly, the COM_0 test achieved the
348 maximum biosorption capacity in both microalgae species. At 72 h, the total biosorption
349 capacity of *C. vulgaris* ranged from 11.9 mg/g in C_{OM0} to 9.2 mg/g in C_{OM3}, whereas that of
350 *S. almeriensis* ranged from 11.2 mg/g in C_{OM0} to 10.4 mg/g in C_{OM3}. The results showed a
351 clear negative influence of the OM concentration on microalgae biosorption capacity for
352 the multimetallic solution under investigation. Nevertheless, correlation of various factors
353 associated with the biosorption process produced a combined effect, which modified the
354 extent of this influence over time (Fig. 4). At higher OM concentration in the SSW, the
355 toxic effect of HM on microalgae decreased. This triggered microalgae growth, which
356 slight increased the pH of the medium. When cellular growth is favored, TOC was
357 subsequently consumed. Therefore, the TOC concentration decreased along the test and the

358 negative effect associated with OM concentration in the biosorption process also decreased.
359 The final differences in the total biosorption capacity observed between COM_0 and COM_3
360 at 72 h can be directly related to the severe effect that OM induced in As biosorption, which
361 was the only toxic element that experienced the interference of OM in the biosorption
362 process over time.



363

364 **Fig. 4.** Schematic correlation of organic matter (OM), cell growth, TOC concentration, and
365 pH medium in the removal capacity of microalgae biomass for a multimetallic system.

366

367 Finally, no precipitation was observed in the multimetallic control samples, probably
368 due to the increase in the solubility of the cationic metals produced by the high borate
369 concentration present in the multimetallic solution (Beckett, 2016; Graff et al., 2017).
370 Future studies should be addressed to analyze possible metabolic responses of microalgae
371 to HM, as the activation of desorption mechanisms to alleviate HM toxicity.

372

373 *3.2. Effect of OM on the* biosorption *of toxic elements from the multimetallic solution with*
374 *CO_2 supply*

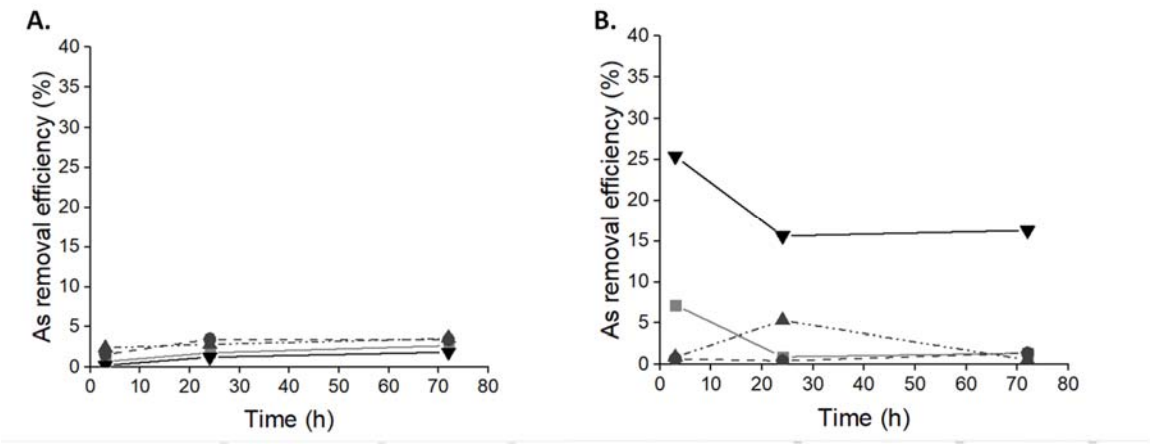
375 The addition of CO_2 in the biosorption experiments resulted in a decrease in the pH
376 from initial neutrality to ≈ 5.2 at 24 h, regardless of the microalgae species. This medium
377 acidification can be directly attributed to CO_2 dissolution in the cultivation broth (Hu et al.,
378 2012). Slight differences in a range of pH of 5.1 – 5.5 were found at 72 h, where the highest
379 pH values were recorded in the test carried out at highest initial OM concentrations.

380 A negative biomass growth was recorded in the *C. vulgaris* biosorption tests conducted
381 with CO_2 addition. The TSS decreased by 10.2% in C_{OM0}, 15.3% in C_{OM1}, 8% in C_{OM2} and
382 4.1% in C_{OM3}. These results suggest a cell decay in cultures mediated by the high
383 concentrations of CO_2 in the medium and by the low pH (<5.5) (Hussain et al., 2017).
384 Interestingly, *S. almeriensis* was able to grow under all conditions tested in spite of the low
385 pH value. Indeed, the TSS concentration increased by 19.4% in C_{OM0} and by 53.1% in
386 C_{OM3} at 72 h. These results confirmed the higher tolerance and growth capacity of *S.*
387 *almeriensis* (compared to *C. vulgaris*) previously found without CO_2 addition.

388 TOC concentrations increased with the time course in *C. vulgaris* experiments. At 72 h,
389 TOC concentrations reached values 676.4%, 348.4%, 179.1%, and 117.7% higher than
390 those observed at time 0 in C_{OM0}, C_{OM1}, C_{OM2}, and C_{OM3}, respectively (Fig. 1C). These
391 findings were likely due *C. vulgaris* decay in excess of CO_2 and the TOC generated can be
392 associated with the activation of protection mechanisms, such as metabolic export
393 processes to control the oxidative stress (Mohamed, 2008). Lower increases in TOC
394 concentrations (327.7%, 232.1% and 133.8% at 72 h in C_{OM0}, C_{OM1} and C_{OM2}, respectively)
395 were recorded for *S. almeriensis* (Fig. 1D). A positive TOC removal efficiency of 34.4%
396 was found in C_{OM3} at 72 h. The moderate increase in TOC concentration, together with the
397 *S. almeriensis* growth observed, could be associated to a partial cellular death but also to

398 the excretion of metabolites, in a lower extent than that observed in *C. vulgaris* (Wang et
399 al., 2010). Interestingly, similar final TOC concentrations of ~ 78 mg/L were detected at 72
400 h in C_{OM1}, C_{OM2}, and C_{OM3} experiments.

401 Arsenic biosorption was severely affected by CO_2 addition, especially in *C. vulgaris*
402 experiments (Fig. 5A), Low As removal efficiencies (< 4 %) were obtained for *C. vulgaris*
403 regardless of the OM in SSW, resulting in a maximum As biosorption capacity of 0.6 mg/g
404 in C_{OM1} at 72 h. CO_2 addition also decreased As removal efficiencies in *S. almeriensis* in
405 SSW with added peptone and meat extract, especially at high contact times ($q_{As,72}$ of 0.06
406 mg/g in C_{OM1}, and 0.1 mg/g in C_{OM2} and C_{OM3}) (Fig. 5B). Only the control experiments
407 supported a significant As biosorption, which decreased from 25.4% at 3 h to 15.6% at 24
408 h, resulting in $q_{As,72}$ of 1.9 mg/g).



409

410 **Fig. 5**. Time course of the removal efficiency (%) of As in biosorption studies with CO_2
411 supply at different initial OM concentrations by *Chlorella vulgaris* (A), and *Scenedesmus*
412 *almeriensis* (B) cultures at 1g TSS/L and pH 7.0. (—▼—): C_{OM0} (control); (- -▲- -): C_{OM1} (40
413 mg peptone /L and 27.5 mg meat extract /L); (– • –): C_{OM2} (80 mg peptone /L and 55 mg
414 meat extract /L), and (—■—): C_{OM3} (160 mg peptone /L and 110 mg meat extract /L).

Similarly, CO_2 addition resulted in even lower B removal efficiencies than those reported in Subsection 3.1 for *C. vulgaris* with $RE_{B,72}$ of ≈ 3 % and a maximum $q_{B,72}$ of 1.8 mg/g in C_{OM3} (Supplementary material). The removal of B was less impacted by CO_2 addition in *S. almeriensis* experiments, with a slight variation of RE_B from 4.0% in C_{OM2} at 3 h to 6.4% in C_{OM0} at 72 h. The final B biosorption capacity of *S. almeriensis* decreased with the OM concentration of SSW from 3.3 mg/g in C_{OM0} to 2.4 mg/g in C_{OM3} (considering cell growth). The lower effect of CO_2 addition on B biosorption studies could be associated with the high solubility of the B complexes in moderately-acidic solutions (Guan et al., 2016). Hence, differences in B removal can be only associated with H_+ competition for the active sites on the surface adsorption process.

Furthermore, CO_2 addition remarkably reduced the Cu, Mn, and Zn removal efficiencies of *C. vulgaris*, with the major impact being in the control tests (Fig. 6A, C, and E). Thus, Cu, Mn, and Zn removal by *C. vulgaris* in C_{OM0} under CO_2 supply at 3 h accounted for 83.5%, 43.4%, and 40.1%, and decreased to 55.2%, 9.3%, and 0.6% at 72 h, respectively. The effect of CO_2 addition was less pronounced in SSW with higher OM concentrations, where RE_{Cu}, RE_{Mn}, and RE_{Zn} remained higher than in the control test, but lower than those recorded in the absence of CO_2 supply. These lower RE of Cu, Mn and Zn could be associated with cell decay mediated by pH modification caused by CO_2 addition. Cu removal at 3 h was the only exception to the general trend, with efficiencies decreasing with the OM concentration of the SSW and values similar or even higher to those obtained without CO_2 addition. The trends in the biosorption capacities were identical to those in the removal efficiencies (increasing with OM) as a result of the decrease in *C. vulgaris*

concentration in this set of experiments. The final biosorption capacities ranged from 2.0 mg/g in C_{OM0} to 2.8 mg/g in C_{OM3} for Cu; from 0.3 mg/g in C_{OM0} to 1.8 mg/g in C_{OM3} for Mn; and from 0.03 mg/g in C_{OM0} to 2.9 mg/g in C_{OM3} for Zn.

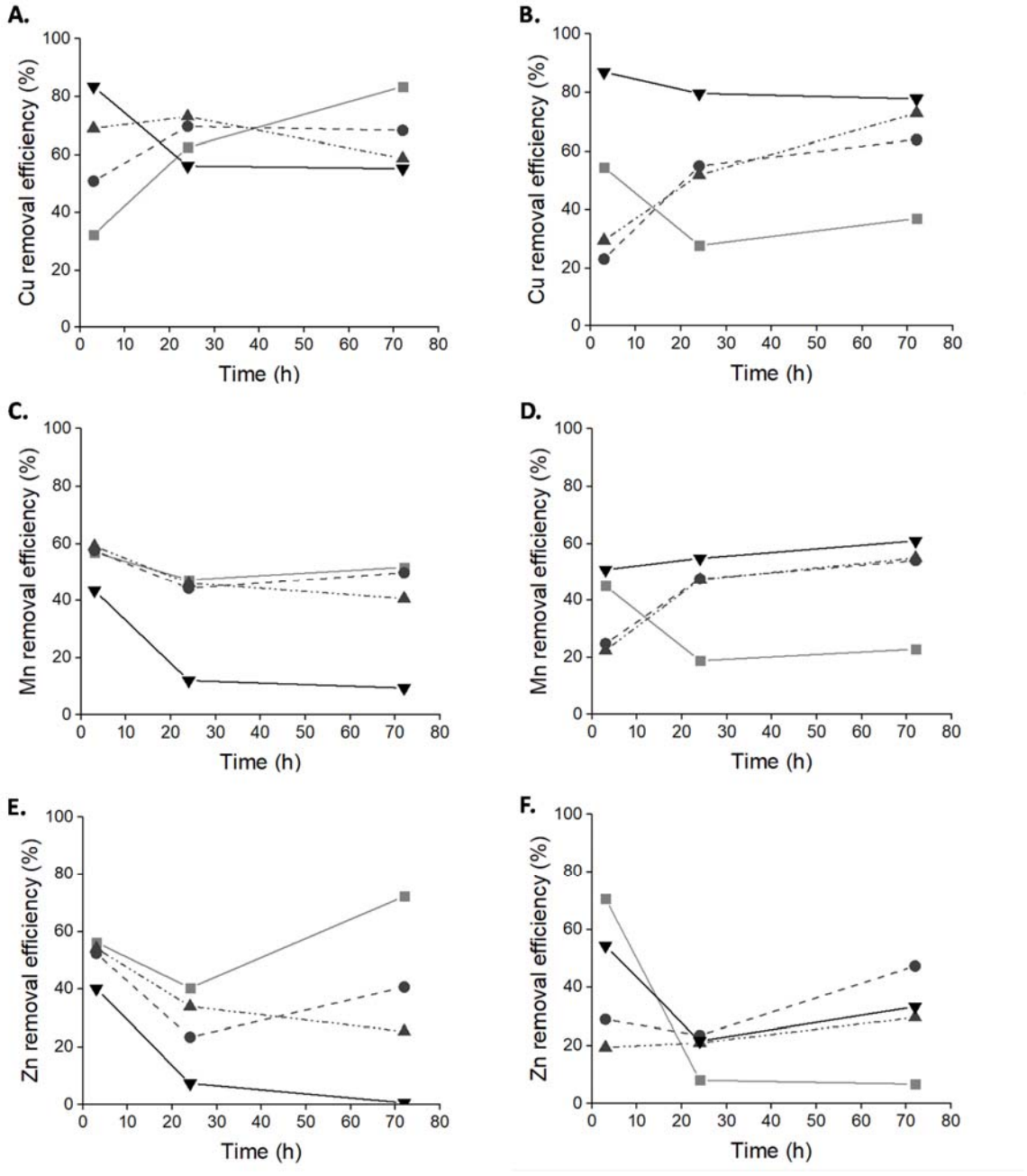


Fig. 6. Time course of the removal efficiency (%) of copper (Cu), manganese (Mn), and zinc (Zn); in biosorption studies with CO_2 supply at different initial OM concentrations by

444 *C. vulgaris* (CV) and by *S. almeriensis* (SA) cultures at 1g TSS/L and pH 7.0. A) CV-Cu;
445 B) SA-Cu; C) CV-Mn; D) SA-Mn; E) CV- Zn; F) SA-Zn. (—▼—): C_{OM0} (control); (- -▲- -):
446 C_{OM1} (40 mg peptone /L and 27.5 mg meat extract /L); (– ● –): C_{OM2} (80 mg peptone /L and
447 55 mg meat extract /L), and (—■—): C_{OM3} (160 mg peptone /L and 110 mg meat extract /L).
448
449 On the other hand, the reduction in Cu, Mn, and Zn removal efficiencies by *S.*
450 *almeriensis* under CO_2 addition increased with the OM concentration of the SSW (Fig. 6B,
451 D, and F). The results at 3 h of contact time represented the only exception, with higher Cu,
452 Mn, and Zn removal efficiencies in C_{OM3} with supply of CO_2. In these tests, the removal
453 efficiencies decreased from 54.6%, 45.0%, and 70.6% at 3 h to 27.8%, 18.8%, and 8.0% at
454 24 h, respectively, with Zn being the toxic element most affected by CO_2 addition
455 regardless of the OM concentration. The Cu, Mn, and Zn biosorption capacities in *S.*
456 *almeriensis* exhibited an inverse correlation with the initial OM concentration, with values
457 decreasing from 2.1 mg/g in C_{OM0} to 0.8 mg/g in C_{OM3} for Cu, from 1.7 mg/g in C_{OM0} to 0.5
458 mg/g in C_{OM3} for Mn, and from 1.0 mg/g in C_{OM0} to 0.2 mg/g in C_{OM3} for Zn. These values
459 were lower than those obtained without CO_2 supply, with the highest decrease being
460 recorded in the C_{OM3} tests.

461 Contrary to the expected effect of stimulation of microalgae growth by CO_2 supply, the
462 results evidenced the negative influence of CO_2 addition on the removal of the target
463 elements by *C. vulgaris* and *S. almeriensis*. The decrease in cell growth was associated to
464 medium acidification, as well as to a metabolic response to the oxidative stress. The
465 increase in OM concentration in the SSW resulted in the opposite effect on the total
466 biosorption capacities in both microalgal species. Thus, whereas the total biosorption

capacity of *C. vulgaris* increased linearly with the initial OM concentrations, from 4.4 mg/g in C_{OM0} to 9.7 mg/g in C_{OM3}, the total biosorption capacity of *S. almeriensis* decreased from 9.7 mg/g in C_{OM0} to 3.9 mg/g in C_{OM3}.

No positive effects on biomass growth or nutrient removal by CO_2 addition were founded in studies of *Chlorella* sp. cultivated in wastewater containing high OM concentrations in a closed photobioreactor system (Min et al., 2011). Indeed, an optimal CO_2 concentration of 6.5% was identified in studies with *Chlorella vulgaris* P12 (Anjos et al., 2013). In contrast, higher growth rates were founded at higher CO_2 concentration (~20%) in cultures of *Scenedesmus* (Tang et al., 2011). Therefore, these results confirmed that high CO_2 concentrations can induce different metabolic pathways between species and determine the OM influence on the overall HM biosorption processes. The differences found between the two pure microalgae species on this study warm about the risk of extrapolating biosorption results of pure species to consortia of different microalgae and bacteria, as those growing in WWT photobioreactors. These results indicate that microalgae could remove efficiently HM from water with low OM content as the river water, but its effectiveness could decrease in WWT photobioreactors with high OM content and, usually, working with CO_2 addition. Further in-situ experiments of HM biosorption in real WWT photobioreactors are required to study the biosorption of toxic elements in these plants. Nevertheless, microalgae could be potentially used as a low-cost complement for the purification step presented in the final stage of the decontamination process.

3.3. Recovery of toxic elements from microalgae

High recovery efficiency yields of As, Cu, Mn, and Zn from *C. vulgaris* (Y_R> 70%)

were obtained with no significant differences ($P > 0.05$) using HCl at 0.1 M and 0.2 M as eluent, regardless of the sampling times (Fig. 7A). The maximum recovery was achieved for As with 0.1 M HCl at 60 min ($Y_{R,As,60}$ of 89 %). Cu, Mn, and Zn recovery increased slightly with the HCl concentration, with maximum Y_R of 81%, 80%, and 78% for Cu, Mn, and Zn, respectively, using 0.2 M HCl at 60 min. A slight increase on Cu recovery efficiency with increasing the HCl concentration from 0.1M (~ 80%) to 0.2M (88%) at 60 min, in studies of *Halimeda gracilis,* was also previously reported (Jayakumar et al., 2015).

By contrast, B recovery from *C. vulg*aris with HCl was low (Y_R of 15-20% regardless of time). HCl at 0.1 M also mediated the highest recoveries of As, Cu, Mn, and Zn from *S. almeriensis,* with an almost complete metal recovery of Mn and Zn (> 99%) at 10 min (Fig. 7B). The recovery of B using HCl from *S. almeriensis* was low regardless of the concentration used. In this context, (Vannela and Verma, 2006) reported the highest recovery efficiencies (> 95%) of Co^{+2}, Cu^{+2}, and Zn^{+2} from S*pirulina platensis* using inorganic acids (0.1 M HCl) in an eluents screening study comparing the performance of inorganic acids, inorganic salts, chelating agents and organic acids. Unexpectedly, the increase in the HCl concentration decreased the recovery efficiencies from *S. almeriensis* with significative differences ($P = 0.001$) for all toxic elements by ~ 50 %, except B, likely due to damage to the cell wall structure caused by the high acid concentration (Abdolali et al., 2015; Kołodyńska et al., 2017).

NaOH at 0.1 M provided the highest As recovery efficiency from *C. vulgaris* (93% at 20 min), a moderate recovery of Zn ($Y_{R,Zn}$ of 40%) and low recovery efficiencies for the rest of the elements tested (Fig. 7C). The increase in the NaOH concentration to 0.2 M improved Zn recovery ($Y_{R,Zn,10}$ of 55%) from *C. vulgaris*, although it slightly decreased the As

513 recovery. Alkaline solutions supported lower recovery efficiencies from *S. almeriensis* than
514 from *C. vulgaris*, with maximum Y_R of 70% for As at 20 min using 0.1 M NaOH, and 51%
515 for Cu at 20 min using 0.2 M NaOH (Fig. 7D). No significant differences ($P > 0.05$) on
516 metal recovery were observed between NaOH concentrations in *S. almeriensis*. The
517 recovery of Mn was low when using NaOH solutions as eluents, with Y_R values lower than
518 8% for both microalgae species.

519 Finally, the recovery efficiencies using $CaCl_2$ increased over time in all the experiments,
520 for all the elements except for B (Fig. 7E, F). The increase of $CaCl_2$ concentration to 0.2 M
521 achieved the maximum recoveries for As and Mn from *C. vulgaris,* reaching Y_R values of
522 of 77% and 72% at 60 min, respectively. On the other hand, a low recovery of all the
523 elements from *S. almeriensis* was obtained. Only, $CaCl_2$ 0.2 M supported a relevant B
524 recovery efficiency of 38% at 10 min, the highest achieved for this element.

525 From the above results, biomass elution with 0.1M HCl supported the best metal
526 recovery performance of the target metals in both microalgae species. Therefore, these
527 conditions were applied during the evaluation of the effect of the OM on the recovery of
528 toxic metals. In this context, a complete recovery of As, Cu, Mn, and Zn using 0.1 M HCl
529 as eluent was achieved in both *C. vulgaris* and *S. almeriensis* loaded with the multimetallic
530 solution in C_{OM3} SSW. The above described decrease in the removal capacity of the
531 biomass induced by the presence of OM in the medium likely facilitated the recovery
532 process. It was noteworthy that kinetics of metal recovery were very fast (data not shown),
533 supporting a total recovery (~100%) of As, Cu, Mn, and Zn within 10 min. The presence of
534 OM in the multimetallic solution used to load the microalgae slightly increased the B

recovery efficiency from *C. vulgaris* (26% at 10 min), but decreased B recovery from *S. almeriensis* (18% at 10 min).

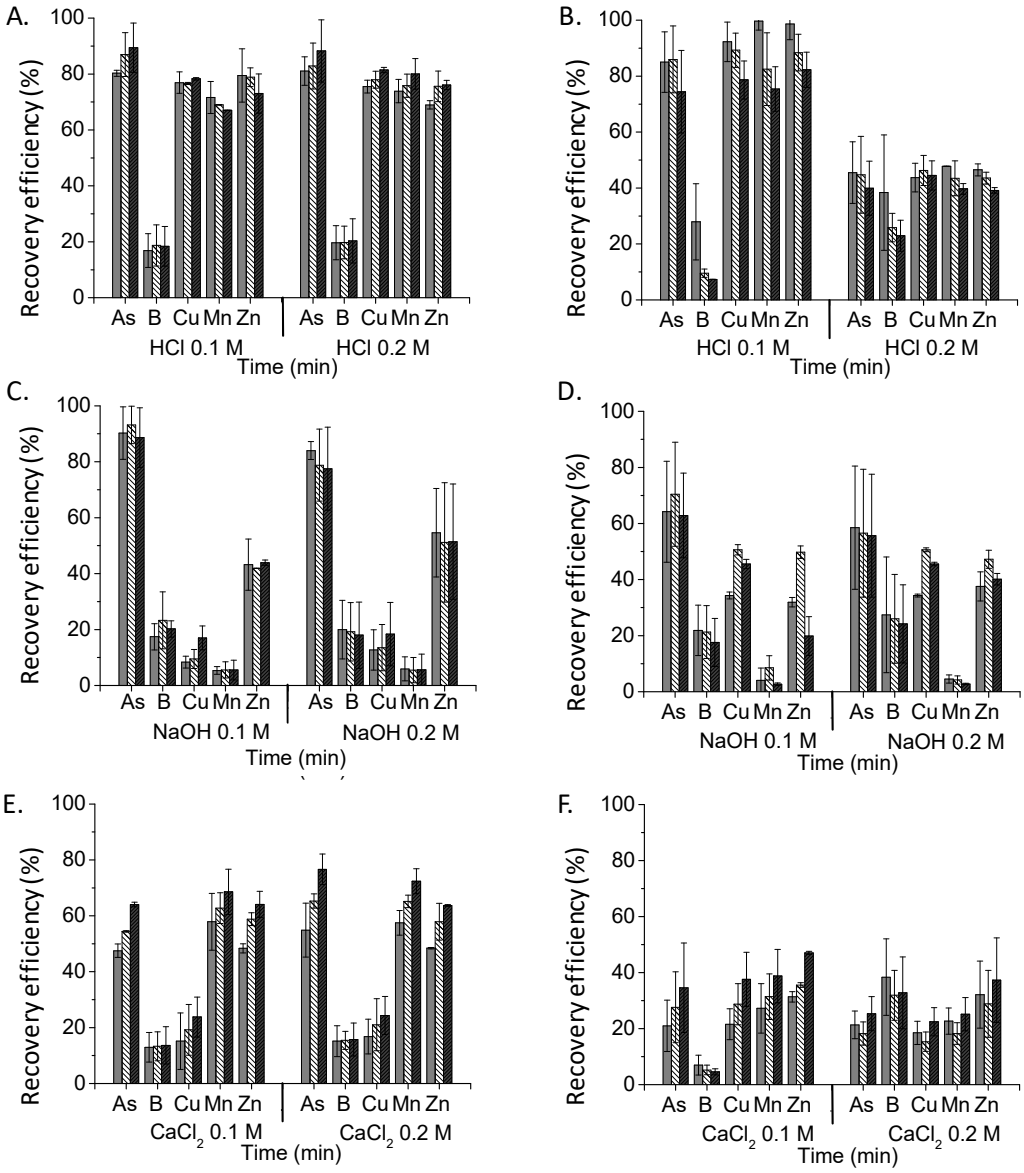


Fig. 7. Time course of the recovery efficiency of toxic elements (%) in *C. vulgaris* (CV), and *S. almeriensis* (SA) cultures at 1g TSS/L and pH 7.0, using different eluents (HCl, NaOH, and $CaCl_2$) at two concentrations (0.1 M, 0.2 M). A) CV – HCl, B) SA – HCl, C) CV – NaOH, D) SA – NaOH, E) CV – $CaCl_2$, F) SA – $CaCl_2$. (■): 10 min, (▨): 20 min, (■): 60 min.

4. Conclusions

S. almeriensis resulted in better performance in HM tolerance, arsenic removal and TOC removal in multimetallic solutions of HM than *C. vulgaris*. A negative effect in RE was associated with OM presence, but this effect decreased along time. The highest arsenic removals (14.9% for CV; 32.4% for SA) were obtained in absence of OM and without CO_2 addition. Boron removal was low (<8.5%), with no clear influence of the presence of OM and CO_2. Overall, the presence of OM decreased Cu, Mn, and Zn removals, but facilitated metal recovery under acidic elution. CO_2 addition decreased the total biosorption capacity from average values of 10.1 and 11.0 mg/g to 7.1 and 6.6 mg/g for CV and SA, respectively. HM biosorption by microalgae can be useful for remediation of water with low OM content, but further in-situ studies of HM biosorption in WWT photobioreactors are recommended. HCl 0.1 M resulted the best eluent for metal recovery, despite supporting low boron recovery.

5. Acknowledgments

This work was supported by the regional government of Castilla y León (UIC 071, CLU 2017-09 and VA080G18). The authors also thank "Ministerio de Ciencia, Innovación y Universidades" (CTQ2017-84006-C3-1-R) and the EU-FEDER (CLU 2017-09 and CTQ2017-84006-C3-1-R) for the financial support of this work. Ricardo Saavedra Concha wishes to thank the National Commission of Science & Technology (CONICYT) of Chile for providing his Doctorate Scholarship (CONICYT – Doctorado Nacional – 21140576).

565

566 **6. References**

567 Abdolali, A., Hao, H., Guo, W., Zhou, J.L., Du, B., Wei, Q., Wang, X.C., Dan, P., 2015.
568 Bioresource Technology Characterization of a multi-metal binding biosorbent :
569 Chemical modification and desorption studies. Bioresour. Technol. 193, 477–487.
570 https://doi.org/10.1016/j.biortech.2015.06.123

571 Abinandan, S., Subashchandrabose, S.R., Pannerselvan, L., Venkateswarlu, K., Megharaj,
572 M., 2019. Potential of acid-tolerant microalgae, *Desmodesmus* sp. MAS1 and
573 *Heterochlorella* sp. MAS3, in heavy metal removal and biodiesel production at acidic
574 pH. Bioresour. Technol. 278, 9–16. https://doi.org/10.1016/j.biortech.2019.01.053

575 Abou-Shanab, R.A.I., Ji, M.K., Kim, H.C., Paeng, K.J., Jeon, B.H., 2013. Microalgal
576 species growing on piggery wastewater as a valuable candidate for nutrient removal
577 and biodiesel production. J. Environ. Manage. 115, 257–264.
578 https://doi.org/10.1016/j.jenvman.2012.11.022

579 Acién, F.G., Fernández, J.M., Magán, J.J., Molina, E., 2012. Production cost of a real
580 microalgae production plant and strategies to reduce it. Biotechnol. Adv. 30, 1344–
581 1353. https://doi.org/10.1016/j.biotechadv.2012.02.005

582 Alcántara, C., Muñoz, R., Norvill, Z., Plouviez, M., Guieysse, B., 2015. Nitrous oxide
583 emissions from high rate algal ponds treating domestic wastewater. Bioresour.
584 Technol. 177, 110–117. https://doi.org/10.1016/j.biortech.2014.10.134

585 Álvarez-Benedí, J., Bolado, S., Cancillo, I., Calvo, C., García-Sinovas, D., 2005.
586 Adsorption–Desorption of Arsenate in Three Spanish Soils. Vadose Zo. J. 4, 282.

https://doi.org/10.2136/vzj2004.0095

Anjos, M., Fernandes, B.D., Vicente, A.A., Teixeira, J.A., Dragone, G., 2013. Optimization of CO_2 bio-mitigation by *Chlorella vulgaris*. Bioresour. Technol. 139, 149–154. https://doi.org/10.1016/j.biortech.2013.04.032

Bădescu, I.S., Bulgariu, D., Ahmad, I., Bulgariu, L., 2018. Valorisation possibilities of exhausted biosorbents loaded with metal ions – A review. J. Environ. Manage. 224, 288–297. https://doi.org/10.1016/j.jenvman.2018.07.066

Beckett, M.A., 2016. Recent advances in crystalline hydrated borates with non-metal or transition-metal complex cations. Coord. Chem. Rev. 323, 2–14. https://doi.org/10.1016/j.ccr.2015.12.012

Bilal, M., Rasheed, T., Sosa-Hernández, J.E., Raza, A., Nabeel, F., Iqbal, H.M.N., 2018. Biosorption: An interplay between marine algae and potentially toxic elements—A review. Mar. Drugs 16, 1–16. https://doi.org/10.3390/md16020065

Cabello, J., Toledo-Cervantes, A., Sánchez, L., Revah, S., Morales, M., 2015. Effect of the temperature, pH and irradiance on the photosynthetic activity by *Scenedesmus obtusiusculus* under nitrogen replete and deplete conditions. Bioresour. Technol. 181, 128–135. https://doi.org/10.1016/j.biortech.2015.01.034

Cameron, H., Mata, M.T., Riquelme, C., 2018. The effect of heavy metals on the viability of *Tetraselmis marina* AC16-MESO and an evaluation of the potential use of this microalga in bioremediation . PeerJ 6, e5295. https://doi.org/10.7717/peerj.5295

Dong, B., Ho, N., Ogden, K.L., Arnold, R.G., 2014. Cultivation of *Nannochloropsis salina* in municipal wastewater or digester centrate. Ecotoxicol. Environ. Saf. 103, 45–53.

https://doi.org/10.1016/j.ecoenv.2014.02.001

Dong, X., Wang, C., Li, H., Wu, M., Liao, S., Zhang, D., Pan, B., 2014. The sorption of heavy metals on thermally treated sediments with high organic matter content. Bioresour. Technol. 160, 123–128. https://doi.org/10.1016/j.biortech.2014.01.006

E.W. Rice, R.B. Baird, A.D. Eaton, editors, 2017. Standard Methods for the Examination of Water and Wastewater, 23rd Edition, American Water Works Association.

El-Sheekh, M.M., Farghl, A.A., Galal, H.R., Bayoumi, H.S., 2016. Bioremediation of different types of polluted water using microalgae. Rend. Lincei 27, 401–410. https://doi.org/10.1007/s12210-015-0495-1

Gani, P., Sunar, N.M., Matias-peralta, H., Latiff, A.A.A., Parjo, U.K., Oyekanmi, A.A., Abdul, A.A., Parjo, U.K., Oyekanmi, A.A., 2017. Green Approach in the Bio-removal of Heavy Metals from wastewaters. MATEC Web Conf. 06007, 1–8. https://doi.org/10.1051/matecconf/20171030 6007

Graff, A., Barrez, E., Baranek, P., Bachet, M., Bénézeth, P., 2017. Complexation of Nickel Ions by Boric Acid or (Poly)borates. J. Solution Chem. 46, 25–43. https://doi.org/10.1007/s10953-016-0555-x

Guan, Z., Lv, J., Bai, P., Guo, X., 2016. Boron removal from aqueous solutions by adsorption - A review. Desalination 383, 29–37. https://doi.org/10.1016/j.desal.2015.12.026

Gupta, V.K., Rastogi, A., 2008. Sorption and desorption studies of chromium (VI) from nonviable cyanobacterium *Nostoc muscorum* biomass. J. Hazard. Mater. 154, 347–354. https://doi.org/10.1016/j.jhazmat.2007.10.032

631 Hamed, S.M., Selim, S., Klöck, G., AbdElgawad, H., 2017. Sensitivity of two green
632 microalgae to copper stress: Growth, oxidative and antioxidants analyses. Ecotoxicol.
633 Environ. Saf. 144, 19–25. https://doi.org/10.1016/j.ecoenv.2017.05.048

634 Hu, B., Min, M., Zhou, W., Li, Y., Mohr, M., Cheng, Y., Lei, H., Liu, Y., Lin, X., Chen, P.,
635 Ruan, R., 2012. Influence of exogenous CO_2 on biomass and lipid acccumulation of
636 microalgae Auxenochlorella protothecoides cultivated in concentrated municipal
637 wastewater. Appl Biochem Biotechnol 166, 1661–1673.
638 https://doi.org/10.1007/s12010-012-9566-2

639 Hussain, F., Shah, S.Z., Zhou, W., Iqbal, M., 2017. Microalgae screening under CO_2 stress:
640 Growth and micro-nutrients removal efficiency. J. Photochem. Photobiol. B Biol. 170,
641 91–98. https://doi.org/10.1016/j.jphotobiol.2017.03.021

642 Jayakumar, R., Rajasimman, M., Karthikeyan, C., 2015. Optimization, equilibrium, kinetic,
643 thermodynamic, and desorption studies on the sorption of Cu (II) from an aqueous
644 solution using marine green algae : *Halimeda gracilis*. Ecotoxicol. Environ. Saf. 121,
645 199–210. https://doi.org/10.1016/j.ecoenv.2015.03.040

646 Jiang, Y., Purchase, D., Jones, H., Garelick, H., 2011. Technical note: Effects of arsenate
647 (as^{5+}) on growth and production of glutathione (GSH) and phytochelatins (PCS) in
648 *Chlorella Vulgaris*. Int. J. Phytoremediation 13, 834–844.
649 https://doi.org/10.1080/15226514.2010.525560

650 Kołodyńska, D., Krukowska, J., Thomas, P., 2017. Comparison of sorption and desorption
651 studies of heavy metal ions from biochar and commercial active carbon. Chem. Eng. J.
652 307, 353–363. https://doi.org/10.1016/j.cej.2016.08.088

653 Lee, H., Nam, K., Yang, J.W., Han, J.I., Chang, Y.K., 2016. Synergistic interaction
654 between metal ions in the sea salts and the extracellular polymeric substances for
655 efficient microalgal harvesting. Algal Res. 14, 79–82.
656 https://doi.org/10.1016/j.algal.2016.01.006

657 Li, Y., Yang, X., Geng, B., 2018. Preparation of Immobilized Sulfate-Reducing Bacteria-
658 Microalgae Beads for Effective Bioremediation of Copper-Containing Wastewater.
659 Water. Air. Soil Pollut. 229. https://doi.org/10.1007/s11270-018-3709-1

660 Marín, D., Posadas, E., Cano, P., Pérez, V., Lebrero, R., Muñoz, R., Blanco, S., 2018.
661 Seasonal variation of biogas upgrading coupled with digestate treatment in an
662 outdoors pilot scale algal-bacterial photobioreactor. Bioresour. Technol. 163, 58-66.
663 https://doi.org/10.1016/j.biortech.2018.04.117

664 Miazek, K., Iwanek, W., Remacle, C., Richel, A., Goffin, D., 2015. Effect of metals,
665 metalloids and metallic nanoparticles on microalgae growth and industrial product
666 biosynthesis: A review. Int. J. Mol. Sci. 16, 23929–23969.
667 https://doi.org/10.3390/ijms161023929

668 Min, M., Wang, L., Li, Y., Mohr, M.J., Hu, B., Zhou, W., Chen, P., Ruan, R., 2011.
669 Cultivating *Chlorella* sp. in a pilot-scale photobioreactor using centrate wastewater for
670 microalgae biomass production and wastewater nutrient removal. Appl. Biochem.
671 Biotechnol. 165, 123–137. https://doi.org/10.1007/s12010-011-9238-7

672 Mirghaffari, N., Moeini, E., Farhadian, O., 2014. Biosorption of Cd and Pb ions from
673 aqueous solutions by biomass of the green microalga, *Scenedesmus quadricauda*. J.
674 Appl. Phycol. 27, 311–320. https://doi.org/10.1007/s10811-014-0345-z

675 Mohamed, Z.A., 2008. Polysaccharides as a protective response against microcystin-
676 induced oxidative stress in *Chlorella vulgaris* and *Scenedesmus quadricauda* and their
677 possible significance in the aquatic ecosystem. Ecotoxicology 17, 504–516.
678 https://doi.org/10.1007/s10646-008-0204-2

679 Montazer-Rahmati, M.M., Rabbani, P., Abdolali, A., Keshtkar, A.R., 2011. Kinetics and
680 equilibrium studies on biosorption of cadmium, lead, and nickel ions from aqueous
681 solutions by intact and chemically modified brown algae. J. Hazard. Mater. 185, 401–
682 407. https://doi.org/10.1016/j.jhazmat.2010.09.047

683 Monteiro, C.M., Fonseca, S.C., Castro, P.M.L., Malcata, F.X., 2011. Toxicity of cadmium
684 and zinc on two microalgae, *Scenedesmus obliquus* and *Desmodesmus pleiomorphus*,
685 from Northern Portugal. J. Appl. Phycol. 23, 97–103. https://doi.org/10.1007/s10811-
686 010-9542-6

687 Palumbo, A.J., Daughney, C.J., Slade, A.H., Glover, C.N., 2013. Influence of pH and
688 natural organic matter on zinc biosorption in a model lignocellulosic biofuel
689 biorefinery effluent. Bioresour. Technol. 146, 169–175.
690 https://doi.org/10.1016/j.biortech.2013.07.053

691 Pérez-Esteban, J., Escolástico, C., Masaguer, A., Vargas, C., Moliner, A., 2014. Soluble
692 organic carbon and pH of organic amendments affect metal mobility and chemical
693 speciation in mine soils. Chemosphere 103, 164–171.
694 https://doi.org/10.1016/j.chemosphere.2013.11.055

695 Piccini, M., Raikova, S., Allen, M.J., Chuck, C.J., 2019. A synergistic use of microalgae
696 and macroalgae for heavy metal bioremediation and bioenergy production through

hydrothermal liquefaction. Sustain. Energy Fuels 3, 292–301. https://doi.org/10.1039/c8se00408k

Posadas, E., Morales, M. del M., Gomez, C., Acién, F.G., Muñoz, R., 2015. Influence of pH and CO_2 source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways. Chem. Eng. J. 265, 239–248. https://doi.org/10.1016/j.cej.2014.12.059

Romero, L., Alonso, H., Campano, P., Fanfani, L., Cidu, R., Dadea, C., Keegan, T., Thornton, I., Farago, M., 2003. Arsenic enrichment in waters and sediments of the Rio Loa (Second Region, Chile). Appl. Geochemistry 18, 1399–1416. https://doi.org/10.1016/S0883-2927(03)00059-3

Rugnini, L., Ellwood, N.T.W., Costa, G., Falsetti, A., Congestri, R., Bruno, L., 2019. Scaling-up of wastewater bioremediation by *Tetradesmus obliquus*, sequential bio-treatments of nutrients and metals. Ecotoxicol. Environ. Saf. 172, 59–64. https://doi.org/10.1016/j.ecoenv.2019.01.059

Saavedra, R., Muñoz, R., Taboada, M.E., Vega, M., Bolado, S., 2018. Comparative uptake study of arsenic, boron, copper, manganese and zinc from water by different green microalgae. Bioresour. Technol. 263, 49–57. https://doi.org/10.1016/j.biortech.2018.04.101

Sahu, A.K., Siljudalen, J., Trydal, T., Rusten, B., 2013. Utilisation of wastewater nutrients for microalgae growth for anaerobic co-digestion. J. Environ. Manage. 122, 113–120. https://doi.org/10.1016/j.jenvman.2013.02.038

Salama, E.S., Kurade, M.B., Abou-Shanab, R.A.I., El-Dalatony, M.M., Yang, I.S., Min, B.,

719 Jeon, B.H., 2017. Recent progress in microalgal biomass production coupled with
720 wastewater treatment for biofuel generation. Renew. Sustain. Energy Rev. 79, 1189–
721 1211. https://doi.org/10.1016/j.rser.2017.05.091

722 Shen, Q.H., Jiang, J.W., Chen, L.P., Cheng, L.H., Xu, X.H., Chen, H.L., 2015. Effect of
723 carbon source on biomass growth and nutrients removal of *Scenedesmus obliquus* for
724 wastewater advanced treatment and lipid production. Bioresour. Technol. 190, 257–
725 263. https://doi.org/10.1016/j.biortech.2015.04.053

726 Suresh Kumar, K., Dahms, H.U., Won, E.J., Lee, J.S., Shin, K.H., 2015. Microalgae - A
727 promising tool for heavy metal remediation. Ecotoxicol. Environ. Saf. 113, 329–352.
728 https://doi.org/10.1016/j.ecoenv.2014.12.019

729 Taştan, B.E., B., Duygu, E., Dönmez, G., Ertit Taştan, B., Duygu, E., Dönmez, G., Taştan,
730 B.E., Duygu, E., Dönmez, G., 2012. Boron bioremoval by a newly isolated *Chlorella*
731 sp. and its stimulation by growth stimulators. Water Res. 46, 167–175.
732 https://doi.org/10.1016/j.watres.2011.10.045

733 Torres, E.M., Hess, D., McNeil, B.T., Guy, T., Quinn, J.C., 2017. Impact of inorganic
734 contaminants on microalgae productivity and bioremediation potential. Ecotoxicol.
735 Environ. Saf. 139, 367–376. https://doi.org/10.1016/j.ecoenv.2017.01.034

736 Vannela, R., Verma, S.K., 2006. Co^{2+}, Cu^{2+}, and Zn^{2+} accumulation by cyanobacterium
737 Spirulina platensis. Biotechnol. Prog. 22, 1282–1293.
738 https://doi.org/10.1021/bp060075s

739 Vijayaraghavan, K., Balasubramanian, R., 2015. Is biosorption suitable for
740 decontamination of metal-bearing wastewaters? A critical review on the state-of-the-

741 art of biosorption processes and future directions. J. Environ. Manage. 160, 283–296.
742 https://doi.org/10.1016/j.jenvman.2015.06.030

743 Wang, L., Wang, Y., Chen, P., Ruan, R., 2010. Semi-continuous cultivation of *Chlorella*
744 *vulgaris* for treating undigested and digested dairy manures. Appl. Biochem.
745 Biotechnol. 162, 2324–2332. https://doi.org/10.1007/s12010-010-9005-1

746 Yamani, J.S., Lounsbury, A.W., Zimmerman, J.B., 2016. Towards a selective adsorbent for
747 arsenate and selenite in the presence of phosphate: Assessment of adsorption
748 efficiency, mechanism, and binary separation factors of the chitosan-copper complex.
749 Water Res. 88, 889–896. https://doi.org/10.1016/j.watres.2015.11.017

750 Yin, K., Lv, M., Wang, Q., Wu, Y., Liao, C., Zhang, W., Chen, L., 2016. Simultaneous
751 bioremediation and biodetection of mercury ion through surface display of
752 carboxylesterase E2 from *Pseudomonas aeruginosa* PA1. Water Res. 103, 383–390.
753 https://doi.org/10.1016/j.watres.2016.07.053

754 Yin, K., Wang, Q., Lv, M., Chen, L., 2018. Microorganism remediation strategies towards
755 heavy metals. Chem. Eng. J. 1–11. https://doi.org/10.1016/j.cej.2018.10.226

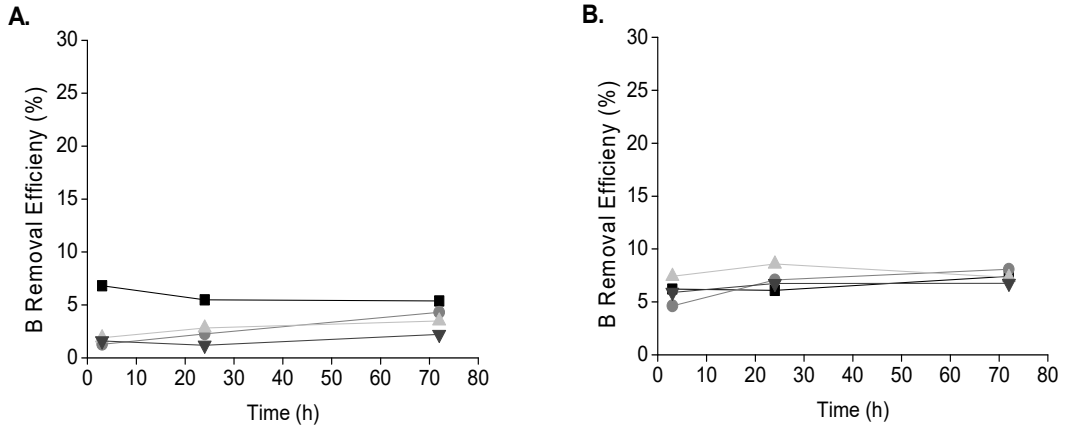
756 Zeraatkar, A.K., Ahmadzadeh, H., Talebi, A.F., Moheimani, N.R., McHenry, M.P., 2016.
757 Potential use of algae for heavy metal bioremediation, a critical review. J. Environ.
758 Manage. https://doi.org/10.1016/j.jenvman.2016.06.059

759 Zhang, J., Hua, P., Krebs, P., 2016. The influences of dissolved organic matter and
760 surfactant on the desorption of Cu and Zn from road-deposited sediment.
761 Chemosphere 150, 63–70. https://doi.org/10.1016/j.chemosphere.2016.02.015

762 Zhang, W., Chen, L., Liu, D., 2012. Characterization of a marine-isolated mercury-resistant

Pseudomonas putida strain SP1 and its potential application in marine mercury reduction. Appl. Microbiol. Biotechnol. 93, 1305–1314. https://doi.org/10.1007/s00253-011-3454-5

Zhang, W., Yin, K., Li, B., Chen, L., 2013. A glutathione S-transferase from *Proteus mirabilis* involved in heavy metal resistance and its potential application in removal of Hg2+. J. Hazard. Mater. 261, 646–652. https://doi.org/10.1016/j.jhazmat.2013.08.023



771

772 **Figure S1.** Time course of the removal efficiency (%) of B in biosorption studies without

773 CO_2 supply at different initial OM concentrations by *Chlorella vulgaris* (A), and

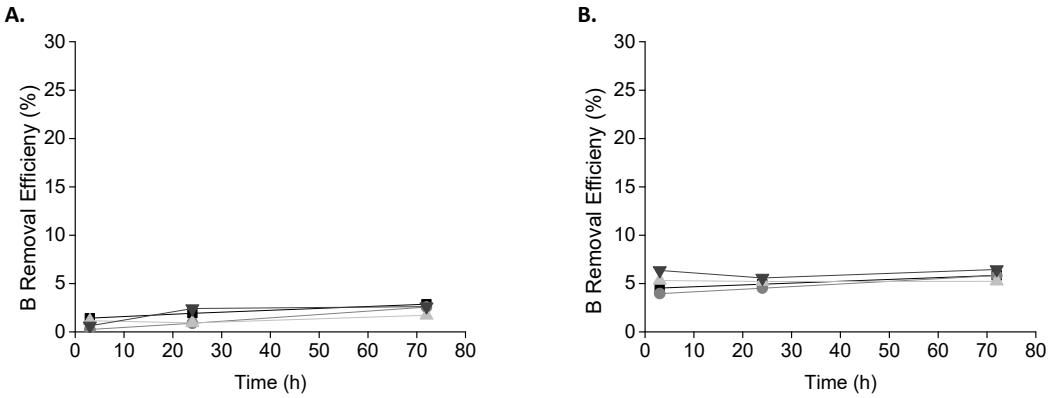
774 *Scenedesmus almeriensis* (B) cultures at 1g TSS/L and pH 7.0. (▼) C_{OM0} (control); (▲)

775 C_{OM1} (40 mg peptone /L and 27.5 mg meat extract /L); (●) C_{OM2} (80 mg peptone /L and

776 55 mg meat extract /L) and (■) C_{OM3} (160 mg peptone /L and 110 mg meat extract /L).

777

778



779

780 **Figure S2.** Time course of the removal efficiency (%) of B in biosorption studies with CO_2

781 supply at different initial OM concentrations in cultures of *Chlorella vulgaris* (A) and

782 *Scenedesmus almeriensis* (B) at 1g TSS/L and pH 7.0. (▼) C_{OM0} (control); (▲) C_{OM1} (40

783 mg peptone /L and 27.5 mg meat extract /L); (●) C_{OM2} (80 mg peptone /L and 55 mg meat

784 extract /L) and (■) C_{OM3} (160 mg peptone /L and 110 mg meat extract /L).

785

786