Nannochloropsis oceanica biomass enriched by electrocoagulation harvesting with promising agricultural applications

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ABSTRACT

Electrocoagulation is a promising technology to harvest and concentrate microalgae while saving costs on secondary dewatering steps. However, the sacrificial electrodes release salts that impact the media and the harvested biomass. This study evaluated the effects of Fe, Zn, and Mg electrodes on *Nannochloropsis oceanica* harvesting and elementary composition of biomass and supernatants. Moreover, plant bioavailability of electrocoagulation minerals attached to biomass was assessed in the tomato plant model *Solanum lycopersicum* (cv. 'Cherry'). Fe electrodes had better performance at lower power consumption and operation costs, followed by Zn and Mg. Electrocoagulation changes biomass and supernatant nutrient composition. Electrodes precipitated Mg and Ca from the nutrient media, enriching *N. oceanica* biomass, but increased Pb 2–4 times and depleted P in supernatants. Finally, Fe and Mg electrode metals in the biomass were proven bioavailable to *S. lycopersicum* seedlings, making electrocoagulation harvested biomass a promising bioresource to agricultural applications.

1. Introduction

Microalgae harvesting is still a bottleneck to its commercial-scale application. Most harvesting technologies are based on mechanical processes, such as centrifugation or filtration (Kadir et al., 2018). While these are viable options to harvest and concentrate microalgae biomass (Roselet et al., 2019), their high maintenance and energy requirements lead to expensive operation (Najjar and Abu-Shamleh, 2020), accounting for 20–30% of the total microalgae production costs (Acién et al., 2017).

Electrocoagulation (EC) is a low-cost technology that can harvest a wide variety of microalgae species with high recovery efficiencies (Landels et al., 2019). During EC, an electrical current is applied through reactive electrodes, commonly made of aluminum (Al) or iron (Fe), that are submerged in the microalgae suspension. The electrode anode suffers electrolytic oxidation, releasing metal ions that act as coagulant agents to microalgae cells, while the generated O_2 and H_2 from water electrolysis promote the flotation of microalgae flocs (Vandamme et al., 2011). The main advantages of electrocoagulation compared to conventional chemical flocculation are: faster harvesting, less water in microalgal flocs (therefore concentrating more the biomass), no pH adjustment requirements (since it performs well at different pH ranges), and the absence of counter ions, such as chloride and sulphate normally present in chemical flocculants (Visigalli et al., 2021). Electrocoagulation has been shown to require less energy than conventional technologies, either for Al (0.06–0.40 kWh/kg) or Fe (0.18–1.79 kWh/kg), which can save energy compared to a one-step centrifugation scenario (Matos et al., 2013; Visigalli et al., 2021). However, electrode corrosion increases harvesting costs and affects biomass quality. Fe electrodes were shown to be more sacrificial than Al ones at the same operation parameters: 1.4–4.6% (w/w) in Fe and 1.0–1.8% (w/w) in Al (Baierle et al., 2015), but 0.6–1.5% of Al were also found in the biomass harvested by EC using Al electrodes (Matos et al., 2013; Vandamme et al., 2011). Nevertheless, while most studies report the concentration of the main corroded material (e.g. Fe^{2+} in biomass from Fe electrodes), the effects of electrocoagulation on biomass and supernatant mineral composition were not yet evaluated.

Not much information can be found in literature on using electrode materials other than Fe or Al to harvest microalgae. Bleeke et al. (2015) studied magnesium (Mg), copper (Cu), and zinc (Zn) electrodes, besides Fe and Al to harvest *Scenedesmus acuminatus*. The highest recovery efficiency (90%) was achieved using Mg, followed by Al, Zn, Cu, and Fe

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electrodes. These authors suggested the use of Mg electrodes for special biomass applications such as food or feed additives, because Mg has a high dietary allowance of 310–400 mg/day (European Commission, 2001). The same is not true for Fe^{2+} and Zn^{2+}, but high concentrations can be decreased by adding a chelate to EC concentrates (Figueiredo et al., 2022). Nevertheless, the present authors suggested non-chelated biomass could still be used for agricultural applications.

Electrocoagulation Fe, Zn, and Mg minerals are essential for plant growth, metabolism and ability to complete its lifecycle (Marschner, 2011). Fe and Zn are both important co-factors for enzymes. Fe helps on the regulation of several metabolic processes, including the biosynthesis of chlorophyll, mitochondrial respiration, nucleotide synthesis, metal homeostasis and the structural conservation of several proteins (Zhang et al., 2019). Zn is involved in pollen formation, antioxidant defense mechanism and contributes to membrane stability and integrity (Abadi and Sepehri, 2015). At last, Mg is greatly known as the central atom of the chlorophyll molecule in the light-absorbing complex of chloroplasts and its function to CO_2 photosynthetic fixation (Cakmak and Yazici, 2010).

Plant deficiencies of Fe, Zn, or Mg are a topic of concern, often associated to soil alkalinity or an extensive and unbalanced fertilization of N, P and K (Guo et al., 2016). The lack of these minerals impacts food production over the world. For example, in the Mediterranean region, it is estimated that 20 to 50% of fruit trees are affected by Fe deficiency, while half of the world soils growing cereal grains are Zn deficient (Alloway, 2009; Jaeger et al., 2000). As for Mg, decreased contents in wheat have been observed after 1968 (Fan et al., 2008). Nutrient deficiencies can be tackled by supplying fortification products, either through soil or foliar fertilization. In the case of Fe, synthetic Fe^{3+} chelates are the most effective and widely used method, though their supplementation can reach 60% of the total fertilization costs (Tagliavini et al., 2000). Also, these are not biodegradable and their frequent use leads to accumulation in aquatic ecosystems, which has serious environmental consequences (Pinto et al., 2014). Microalgae biomass fertilization is already a very effective strategy to promote plant growth and stress resistance (Ferreira et al., 2021). Thus, if EC metals bound to microalgae cells are bioavailable to plants, the biomass could also serve as a substitute for synthetic chelation, being a sustainable strategy to overcome deficits in plant nutrition.

In this work, electrocoagulation using Fe, Zn, and Mg electrodes was used to harvest *Nannochloropsis oceanica* biomass and evaluate its effects on the mineral composition of both EC resultant microalga biomass and supernatants. The EC harvested biomass was then used to assess if cell-coagulated metals are bioavailable to *Solanum lycopersicum*, expanding the possibility of using EC biomass for agricultural applications.

2. Materials and methods

2.1. Culture growth conditions

Nannochloropsis oceanica 0011NN from Allmicroalgae S.A. (Pataias, Portugal) culture collection was cultivated in a 25 m³ horizontal tubular photobioreactor with adjusted Guillard's f/2 medium supplemented with 10 mM of nitrate. It was maintained under a summer circadian photoperiod (39.652897098, −8.990106363) with temperature kept below 30 °C using a sprinkling water system and pH adjusted from 7.5 to 8.0 with an automated CO_2 injection system. The culture was collected at late exponential growth with a concentration of ±3 g/L.

2.2. Electrocoagulation (EC) experiments

EC trials were performed in batch mode using 600 mL glass flasks holding 500 mL of *N. oceanica* culture. Electrode plates were separated by 1 cm distance and connected to an external direct current power source (HY3005D, Mastech, USA; Fig. 1a). The electrode materials used were Fe, Zn and Mg with 100 mm × 45 mm in size (Fig. 1b) and 20 cm² of submerged surface area.

The current was fixed before operation with the controllable power source, while voltage was varied automatically by the equipment. Culture pH was adjusted before operation using HCl and NaOH (1 M). Previous optimized time, pH, and current density for each electrode material (Figueiredo et al., 2022) were used to harvest *N. oceanica* (Table 1).

The microalga suspension was stirred at 150 rpm and left to settle after EC for 30 min. From this point on, the settled culture is referred as concentrate, while the upper phase containing media is referred as supernatant. Concentrates were then centrifuged (4000 ×*g* for 5 min at 4 °C) and freeze dried for analyses.

2.3. Evaluation of electrocoagulation performance

The performance of EC was assessed by recovery efficiency and concentration factor according to Figueiredo et al. (2022). As for power

Table 1
Values of pH, time, and current density used for Fe, Zn and Mg electrocoagulation (Figueiredo et al., 2022).

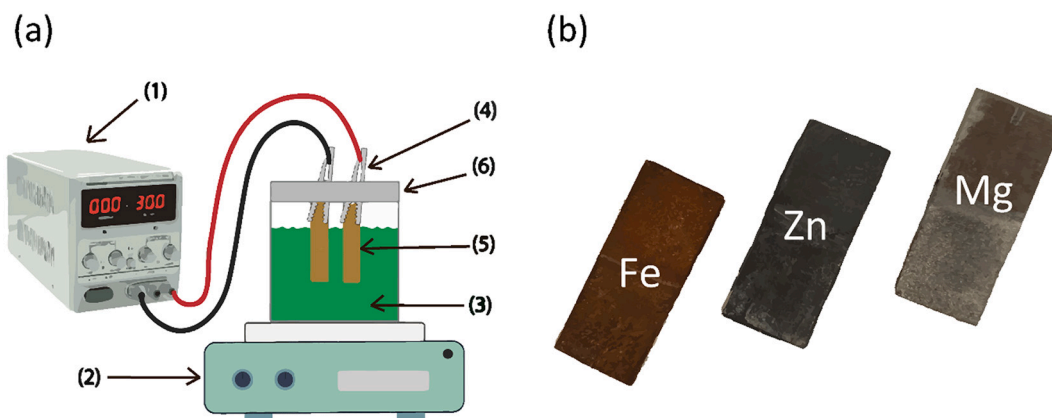| Electrode material	pH	Time (min)	Current density (mA/cm²)
Fe	8.0	7.7	49.2
Zn	7.0	8.9	95.5
Mg	6.5	12.8	100.0

Fig. 1. Schematic representation of the experimental set-up for electrocoagulation (a): Direct current power source (1), magnetic stirrer (2), microalgae culture (3), electrical wires and alligator clips (4), electrode plates (5) and a stabilizing cap (6); (b): Electrode plates of Fe, Zn, and Mg (Figueiredo et al., 2022).

consumption, it was calculated through Eq. (3) according to Vandamme et al. (2011):

$$Power\ consumption\ (kWh/kg\ DW) = \frac{U \times I \times t}{1000 \times V \times \eta_a \times c_i} \quad (1)$$

where U is the voltage (volts), and I is the current (Amps) displayed by the DC power supply during EC operation time; t is the operation time (h); V is the volume of the microalgae suspension (m^3); η_a is the recovery efficiency and c_i is the initial culture concentration (kg/m^3).

To determine EC operation costs, both power consumption and electrode corrosion costs must be calculated. Electrode corrosion was assessed considering the biomass metals from EC and by the Faraday's law (Eq. (2)), which predicts the electrode corrosion mass (grams) that is lost during the electrolysis process.

$$Electrode\ corrosion = \frac{I \times t \times M}{n \times F} \quad (2)$$

The corroded mass W (grams) is equal to the current intensity (I; Amps) multiplied by time (t; seconds) and by the molar weight of the electrode material (M; g/mol). These are then divided by the number of electrons exchanged (n), which is 2e$^-$ for the case of Fe^{2+}· Zn^{2+} and Mg^{2+}, multiplied by the Faraday constant (F; 96485 C/Mol).

Operation costs of EC, shown as price per mass of harvested biomass were determined using Eq. (3):

$$Operation\ costs\ (€/kg) = E \times Ce + Mb \times Cm \quad (3)$$

where E is the power consumption per mass of harvested microalga (kWh/kg), Ce the unit price of industrial electricity cost (0.137 €/kWh in Portugal in 2020), Mb is the mass of corroded metal per mass of biomass (g/kg) and Cm is the cost of the electrode material used to harvest one kg of biomass (€/kg). Electrode material costs were estimated by provided budgets of local companies.

2.4. Mineral composition of EC harvested biomass and supernatant

Supernatant and freeze-dried biomass concentrates obtained from Fe, Zn and Mg EC were analyzed for macronutrients and micronutrients, as well as human-potentially toxic elements. For this, biomass samples of 100 mg were first digested in 5 mL of HNO$_3$ (>68% trace analysis grade) and were left to react for approximately 20 min in a fume hood. The tubes were placed in the autosampler rack of the Microwave (Discover SP, Microwave Synthesizer CEM Corporation). The digestion requirements for each sample were as follows: 3 min time lap to reach 200 °C, followed by steady digestion for 3 min at the same temperature and 2 min time to cool down. A maximal pressure was set to 300 psi and power to 300 W. Samples were diluted to a final volume of 10 mL with Milli-Q-H$_2$O. Blanks samples were performed using the same digestion process with 6 mL of HNO$_3$ (without sample).

Mineral analysis was conducted in the residues diluted in 5% HNO$_3$ in a Microwave Plasma-Atomic Emission Spectrometer (MP-AES; Agilent 4200 MP-AES, Agilent Victoria, Australia). Calibration curves were conducted for each mineral using certified standard solutions from Agilent Technologies. Limits of detection (LOD) and the limits of quantification (LOQ) were calculated based on the standard deviation and slope of the calibration curves. Biomass samples were analyzed in biological triplicates and blanks were subtracted to correct the final mineral concentrations.

2.5. Evaluation of the bioavailability of electrocoagulation metals in plant seedlings growth

Certified seeds of *Solanum lycopersicum* L. cv. 'Cherry' were acquired from Semillas Fitó (Barcelona, Spain) and randomly placed in 9.5 cm Petri dishes containing two Whatman no. 1 filter papers soaked in distilled water. Seeds were germinated at laboratory room temperature

using undirect natural light. After two weeks seedlings with at least one fully expanded leaf were transferred to individual 15 mL tubes containing ¼ strength Hoagland's solution adjusted for each tested condition. Microalga harvested using the different electrodes (Fe, Zn, and Mg) was added to Hoagland solution deficient in the corresponding mineral, to test whether *S. lycopersicum* could utilize the microalga EC metals. The positive control was the ¼ strength Hoagland complete nutrient solution, with the following composition (in mM): 1.25 Ca(NO$_3$)$_2$·4H$_2$O, 1.25 KNO$_3$, 0.25 KH$_2$PO$_4$, 0.5 MgSO$_4$·7H$_2$O and (in µM): 11.5 H$_3$BO$_3$, 0.2 ZnSO$_4$·7H$_2$O, 0.1 CuSO$_4$.5H$_2$O, 2.25 MnCl$_2$·4H$_2$O, 0.005 (NH$_4$)$_6$Mo$_7$O$_{27}$·H$_2$O and 2.5 of Fe (III) chelated by ortho-ortho EDDHA. Seedlings grown in individual Fe, Zn and Mg deficient Hoagland solutions without microalga supplementation were used as negative controls. At last, a third control was used to evaluate the effect of the microalga, by cultivating seedlings in complete Hoagland supplemented with *N. oceanica* harvested solely by centrifugation. Information of sample groups, plant cultivation media, microalga biomass and concentrations used, are presented in Table 2. Microalga biomass harvested using EC was added to supplement Fe, Zn, and Mg metals at equal concentrations of the full-strength Hoagland solution to avoid limiting concentrations of these metals (e.g. due to biomass sedimentation). The pH of the Hoagland solutions was adjusted to 6.0 ± 0.1 using NaOH and HCl 0.1 M, and their electrical conductivity was measured using a conductivity meter (LF95/SET, WTW). Seedlings were randomly placed in a plant growth chamber (ARALAB PLV600, Portugal) and grown at 25 °C for 13 days with photosynthetic active radiation (PAR) of ±125

Table 2
Experimental treatments used for testing the bioavailability of Fe, Zn and Mg electrode metals. Algal biomasses containing electrode metals were added to dHo + EC$_{Fe}$/EC$_{Zn}$/EC$_{Mg}$ samples to fit the respective metal concentration of Hoagland solutions.

Sample	Media	Added algal biomass (mg/L)	Metal concentration (µM)	Conductivity (dS/m)
dHo$_{Fe}$	Fe deficient Hoagland	0.0	0.0	0.43
dHo$_{Zn}$	Zn deficient Hoagland	0.0	0.0	0.43
dHo$_{Mg}$	Mg deficient Hoagland	0.0	0.0	0.39
cHo	Complete Hoagland	0.0	2.5 Fe^{2+}, 0.2 Zn^{2+}, and 500 Mg^{2+}	0.41
dHo + EC$_{Fe}$	Fe deficient Hoagland + biomass after Fe EC	5.8	10.0 (Fe^{2+})	0.40
dHo + EC$_{Zn}$	Zn deficient Hoagland + biomass after Zn EC	0.2	0.8 (Zn^{2+})	0.37
dHo + EC$_{Mg}$	Mg deficient Hoagland + biomass after Mg EC	338.1	2000.0 (Mg^{2+})	1.19
cHo + B$_C$	Complete Hoagland + centrifuged biomass at EC$_{Fe}$ concentration	5.8	2.6 (Fe^{2+})	0.37
	Complete Hoagland + centrifuged biomass at EC$_{Zn}$ concentration	0.2	0.2 (Zn^{2+})	0.42
	Complete Hoagland + centrifuged biomass at EC$_{Mg}$ concentration	338.1	569.5 (Mg^{2+})	0.57

μmol/m^2.s^1 and a light/dark cycle of 16 h/8 h. Decreasing volume in the experimental tubes was avoided by adding demineralized water along with nutrition solutions replacements when electrical conductivities achieved 0.1–0.2 dS/m.

2.5.1. Evaluation of plant chlorophyll content and growth

Effects of nutrient deficiencies were evaluated by estimating the degree of chlorosis (decrease of chlorophyll) in leaves, as it is a common first symptom of nutrient deficiency. Chlorophyll was quantified using a SPAD-502 apparatus (Konica Minolta, Japan). SPAD readings were performed in triplicate in both apical and basal leaves on days 8, 10 and 13 for each plant and values were converted into total chlorophyll concentration (μmol/m^2) using the calibration curve ($r^2 = 0.97$; $n = 34$; $p > 0.001$) for Cherry tomato (Eq. (4)):

$$Total\ Chl\ \left(\mu mol/m^2\right) = 0.16 + SPAD^2 + 18.67*SPAD \qquad (4)$$

The calibration curve was done using leaf discs collected with different degrees of chlorosis and where SPAD readings were carried out, and pigments were extracted using pure acetone in the presence of sodium ascorbate. The absorbance of the samples was measured spectrophotometrically (ABADÍA and ABADÍA, 1993; Litchtenthaler, 1987). Plant growth was evaluated by the dry weights of shoots (stems + leaves) and roots, which were dried at 60 °C (Memmert UM500) for at least 24 h.

2.6. Statistics

One-way ANOVA was used to describe the effects of EC electrodes on recovery efficiency, concentration factor and power consumption and costs to harvest *N. oceanica* biomass. Effects of EC electrodes on the mineral concentration of *N. oceanica* biomass and EC supernatants was also assessed using One-way ANOVA, as well as to describe the effects of different Hoagland solutions on the *S. lycopersicum* dry weight and chlorophyll contents. For all tests, the *p*-values resulting from the sum of square analyses were used to describe the impact of the factors, while Tukey's post hoc test was used to detect differences among treatments. A significance level (α) of 0.05 was considered for all tests.

3. Results and discussion

3.1. Electrocoagulation performance

Figueiredo et al. (2022) showed that Fe, Zn, and Mg electrodes can successfully harvest *N. oceanica* biomass with recovery efficiencies from 96.9 to 98.5% (Fig. 2a) and more relevant differences were found in concentration factors (2.8–7.2; $p < 0.05$; Fig. 2b).
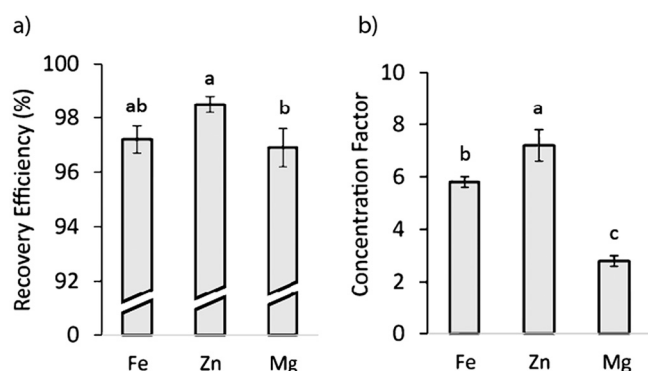
High recovery efficiencies were also reported before using Fe, Mg and Zn electrodes (Bleeke et al., 2015), though concentration factors were not measured by the authors. Mg electrodes concentrated less the biomass, compared to Fe or Zn, showing a decreased capacity to harvest *N. oceanica.* Differences in concentration factors can be due to varying electrode densities, since heavier metals (Zn > Fe > Mg) form denser microalgae-metal flocs, which increase the settling velocity (Khelifa and Hill, 2006).

Electrode materials showed significant differences in power consumption to harvest 1 kg of dried *N. oceanica* (Fe = 0.75 kWh/kg, Zn = 1.26 kWh/kg, Mg = 4.71 kWh/kg; $p < 0.05$). The lower power consumption in Fe electrodes is related to these electrodes requiring less time and current, compared to Zn and Mg electrodes (Table 1). Contrary, higher power consumption in Mg compared to Zn can be explained by the longer time and lower recovery efficiencies. Electrical conductivity in metals could also affect power consumption since electrodes with higher conductivities need less voltage to supply the same amount of current, leading to lower power consumed.

Different power consumption results have been reported in the literature. While 2 kWh are required to harvest a kilogram of *Chlorella vulgaris*, only 0.3 kWh are needed for *Phaedactylum tricornutum* (Vandamme et al., 2011) with Al electrodes. For the same electrodes, 0.24 kWh/kg was only required for a complete removal of *Nannochloropsis* sp. (Matos et al., 2013), while Uduman et al. (2011) reported 9.16 kWh to harvest *Tetraselmis* sp. and *Chlorococcum* sp. using Al and Fe electrodes. These differences confirm the dependency of EC performance on several parameters such as microalgae species, culture's state, time, pH, among others.

EC operational costs accounted both electricity and electrode substitution costs based on electrode corrosion predictions. Fe electrodes had the lowest operational cost according to Faraday estimation, followed by Zn, and Mg (Fe = 0.27 €/kg, Zn = 0.56 €/kg, Mg = 3.85 €/kg; Fig. 3b). While the same price order is true for MP-AES calculations, lower operation costs were found compared to Faraday (Fe = 0.16 €/kg, Zn = 0.30 €/kg, Mg = 1.80 €/kg). Metal contents found in biomass were lower than the Faraday estimations for electrode corrosion, which suggest overestimations for the latter. In fact, Faraday predictions yield (ϕ), which is used to account the gap between theoretical and experimental dissolution of the anode, is usually lower than 1 (Hakizimana et al., 2017), therefore tending to overestimate experimental results. Furthermore, Mg electrodes had aggravated costs compared to power consumption results, which is due to Mg being more expensive than Fe or Zn (Mg = 8.33 €/kg; Zn = 0.56 €/kg, Fe = 0.62 €/kg). Hence, Mg electrodes may not be attractive option to harvest microalgae for low-
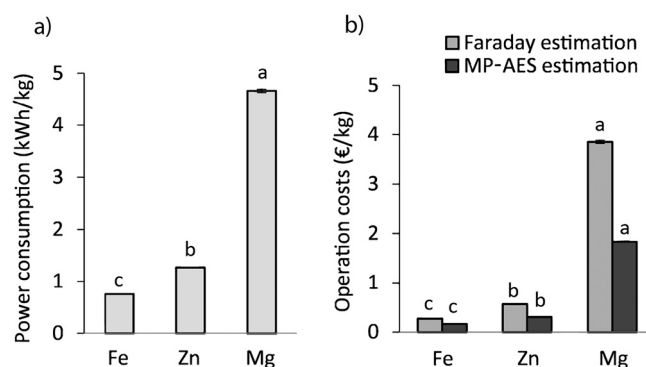
Fig. 2. Effect of Fe, Zn and Mg electrodes on electrocoagulation recovery efficiency (a) and concentration factor (b) (Figueiredo et al., 2022). Different letters indicate significant differences among electrocoagulation electrodes (Tukey's test, $p < 0.05$) and data are shown as mean ± standard deviation ($n = 3$).

Fig. 3. Effect of Fe, Zn and Mg electrodes on electrocoagulation power consumption (a); and harvesting operation costs (b). Electrode corrosion used to calculate operation costs was estimated from the Faraday equation and electrode metals in biomass from the Microwave Plasma-Atomic Emission Spectrometer (MP-AES) results. Different letters indicate significant differences among electrocoagulation electrodes (Tukey's test, $p < 0.05$) and data are shown as mean ± standard deviation ($n = 3$).

cost applications.

3.2. Effects of Fe, Zn, and Mg electrocoagulation on the mineral composition of harvested biomass and supernatants

Effects of EC on the mineral composition of biomass and supernatants were studied by comparing electrodes (Fe, Zn, Mg) and centrifugation samples (Table 3). Results show EC biomasses were not enriched with potassium (K) but supernatants showed decreased concentrations ($p < 0.05$). K is water-soluble but can precipitate by reacting with other compounds, such as K-struvite ($MgKPO_4 \cdot 6H_2O$) (Hu et al., 2020). These molecules are not believed to be involved in coagulation processes (Reyerson et al., 1947), therefore not attaching to biomass. Contrary, contents of Ca significantly increased in all EC biomasses (4–7 times; $p < 0.05$), while Mg increased in biomasses of Zn and Mg EC (3 and 29 times, respectively; $p < 0.05$). Lower contents found in Mg and Ca supernatants suggest these compounds precipitated, which could be related to increased pH (Smith and Davis, 2012). Indeed both Mg and Ca form precipitates at alkaline pH (e.g. $MgOH$ or $Ca_3(PO_4)_2$) that have been associated to microalgae harvesting (Beuckels et al., 2013; Vandamme et al., 2014). As for P, its content in EC biomasses was not different from only centrifuged ($p > 0.05$) but it strongly decreased in all supernatants (3–11 times; $p < 0.05$). EC is known as highly efficient to decrease P in wastewater (Mores et al., 2016), with Fe or Al electrodes reported to remove orthophosphate 8 to 9 times faster than Mg electrodes (Devlin et al., 2019). Contrary, more prominent removals of P were found in this study for Mg electrodes (21.7 to 1.6 mg/L), which again could be due to $MgKPO_4$ crystallization. Indeed, this is suggested by high contents of Mg and K in the culture media and their decrease in supernatants. As for Fe, all EC electrodes decreased their supernatant content to undetected concentrations (Fe < 0.80 mg/L), probably precipitating as hydroxide species ($Fe_x(OH)x$) or ferric phosphate ($FePO_4$) (Dalton et al., 1983; Noubactep and Schöner, 2010). Contrarily, Zn was found at a higher concentration in the supernatant left by Zn electrodes (5-fold increase; $p < 0.05$), which is a result of electrode corrosion. As previously reported, sacrificial electrodes (Fe, Zn, and Mg) resulted in very high metal concentrations in biomass: 95.5 mg Fe^{2+}/g using Fe electrodes; 229.8 mg Zn^{2+}/g using Zn electrodes; 143.8 mg Mg^{2+}/g using Mg electrodes (Figueiredo et al., 2022).

Increased contents of Cu were found in biomass harvested using Zn electrodes (26 times, $p < 0.05$) and no significant differences were found in supernatants ($p > 0.05$). As for Mn, it was higher in biomasses from Fe and Mg electrodes (5–9 times, $p < 0.05$) and was only detected in the supernatant left by Fe electrodes (0.2 mg/L). These mineral increases in biomasses suggest both Cu and Mn cations were involved in cell coagulation (Eslami et al., 2019), but also indicate nutrient imbalances, as increased contents in biomasses were not accompanied by nutrient decreases in supernatants. This could be due to electrode impurities, as

according to Zn electrode's supplier, Cu was 0.17% of the total electrode mass, explaining its 26-fold increase in biomass from Zn EC. Elevated contents of Mn in Fe biomass are expected due to Fe electrodes having Mn in its composition, as it is used to remove oxygen and sulfur in Fe and steel manufacturing (Cannon, 2014). Enriched and bioavailable Cu and Mn contents may be an added benefit by reusing the supernatant for new culturing media, as these are essential nutrients for microalgae growth. On the opposite side, microelement analyses suggest P and Fe^{2+} should be supplemented after all EC electrodes, while Zn^{2+} concentrations from Zn electrodes need to be monitored to avoid reaching toxic concentrations.

3.3. Effects of Fe, Zn, and Mg electrocoagulation on the presence of potentially toxic metals in harvested biomass

Potential toxic compounds Pb, Ni, As, Cr and Cd were quantified in *Nannochloropsis oceanica* biomasses resultant from EC using Fe, Zn, and Mg electrodes and only centrifuged biomass (Table 4). All electrodes increased Pb concentrations 2–3 times ($p < 0.05$) compared to non-EC biomass. Other elements, such as As and Cr were electrode dependent, as increases were only observed using Fe electrodes ($p < 0.05$). Cd was only detected in biomass harvested using Fe electrodes (Cd = 0.79 μg/g, $Cd_{LOD} = 0.54$ μg/g), while Ni was not detected ($Ni_{LOD} = 0.86$ μg/g) in any biomass.

Toxic metal contents in harvested EC biomasses can have different sources. For instance, increased contents of Pb in EC biomasses could be related to electrode impurities but also to alkaline pH. Pb forms positively charged hydroxide precipitates from pH 8 to 11 (Nikolaychuk, 2018), which seem to attach the microalga cell wall. As for Pb contents already in non-EC biomass (6.2 μg/g), it could be related to *N. oceanica* accumulating this heavy metal. Bioremediation studies have been reporting *Nannochloropsis* sp. as a heavy metal accumulator (Leong and Chang, 2020) and *Nannochloropsis oculata* has been successfully used to treat Pb compromised waters (Waluyo et al., 2020). Increased As, Cr, and Cd contents only in Fe electrodes biomass suggest a high level of

Table 4
Effects of Fe, Zn, and Mg electrocoagulation on the concentration of potentially toxic metals in harvested biomass. Different letters indicate significant differences among electrocoagulation electrodes (Tukey's test, $p < 0.05$) and data are shown as mean ± standard deviation ($n = 3$). ND – not detected.

Harvesting	Pb	Ni	As	Cr	Cd
	μg/g				
Centrifuge	6.2 ± 1.0^c	ND	0.1 ± 0.0^b	0.6 ± 0.2^{bc}	ND
Fe EC	13.0 ± 1.4^b	ND	12.4 ± 1.2^a	5.4 ± 0.1^a	0.8 ± 0.1
Zn EC	14.6 ± 1.4^{ab}	ND	0.1 ± 0.0^b	1.0 ± 0.1^b	ND
Mg EC	20.8 ± 3.4^a	ND	0.1 ± 0.0^b	0.4 ± 0.1^c	ND

Table 3
Effects of Fe, Zn, and Mg electrocoagulation on the mineral composition of harvested biomass and supernatants. Different letters indicate significant differences among electrocoagulation electrodes (Tukey's test, $p < 0.05$) and data are shown as mean ± standard deviation ($n = 3$). ND – not detected.

	Harvesting	K	Mg	Ca	P	Fe	Zn	Cu	Mn
		mg/g						μg/g	
Biomass	Centrifuge	7.5 ± 2.2^a	5.0 ± 0.6^c	1.2 ± 0.3^c	10.0 ± 0.7^a	0.6 ± 0.2^b	0.01 ± 0.0^b	4.0 ± 1.6^b	32.0 ± 7.7^c
	Fe EC	3.8 ± 1.3^a	9.8 ± 1.4^c	5.2 ± 0.1^b	12.2 ± 2.7^a	95.5 ± 5.7^a	0.1 ± 0.0^b	20.0 ± 11.3^b	297.4 ± 45.3^a
	Zn EC	7.2 ± 3.6^a	15.4 ± 2.4^b	5.9 ± 1.0^b	8.8 ± 1.8^a	0.7 ± 0.1^b	229.8 ± 21.7^a	104.1 ± 2.9^a	50.3 ± 1.8^c
	Mg EC	9.0 ± 2.0^a	143.8 ± 1.6^a	8.6 ± 1.2^a	11.8 ± 1.0^a	0.4 ± 0.1^b	0.03 ± 0.0^b	5.9 ± 0.7^b	189.4 ± 14.6^b

	Harvesting	K	Mg	Ca	P	Fe	Zn	Cu	Mn
		mg/L							
Supernatant	Centrifuge	652.5 ± 50.7^a	680.2 ± 43.0^a	126.5 ± 13.5^a	21.7 ± 3.6^a	1.3 ± 0.4	0.1 ± 0.0^b	0.2 ± 0.0^a	ND
	Fe EC	383.6 ± 112.0^b	553.5 ± 37.4^b	96.7 ± 21.6^a	5.8 ± 1.0^b	ND	0.1 ± 0.0^b	0.1 ± 0.1^a	0.2 ± 0.0
	Zn EC	574.6 ± 11.4^{ab}	553.2 ± 27.0^b	110.7 ± 11.8^a	2.1 ± 0.6^b	ND	0.5 ± 0.1^a	0.2 ± 0.0^a	ND
	Mg EC	500.2 ± 42.9^{ab}	549.5 ± 19.5^b	84.9 ± 0.9^a	1.6 ± 0.4^b	ND	0.1 ± 0.0^b	0.2 ± 0.0^a	ND

impurities for these electrodes. Selecting purer electrode plates is therefore important to avoid the accumulation of potentially toxic metals.

3.4. Bioavailability of microalga-coagulated Fe, Zn, and Mg EC metals for Solanum lycopersicum growth

Biomasses of *Nannochloropsis oceanica* enriched with electrode metals were added to a metal deficient Hoagland solution (e.g. biomass from Fe-EC added to a nutrient solution deficient in Fe^{2+}) to evaluate if EC coagulated metals can be taken up and utilized by *S. lycopersicum* (Fig. 4; Fig. 5). Shoots and roots from plants grown in Fe deficient nutrient solution (dHo) had a lower weight than plants grown in complete nutrient solution (cHo, $p < 0.05$; Fig. 4a). Also, chlorophyll results showed lower contents in dHo plants compared to cHo, with differences increasing from 423 $\mu mol/m^2$ at day 8 to 606–609 $\mu mol/m^2$ at day 10

and 13, respectively. As expected, the detrimental effects on plant development were due to Fe being an essential element in numerous metabolic processes (Zhang et al., 2019). Hence, Fe concentration and its distribution in plant organs and tissues must be very well balanced and within the physiological limits to ensure it is not limiting metabolic processes and consequently, plant survival (Guerinot and Yi, 1994). The supplementation of Fe EC biomass (dHo + EC_{Fe}) improved the shoot and root weights compared to the respective deficient solution (Fe dHo; $p < 0.05$), with no differences compared to cHo ($p > 0.05$) and no observed morphological stress (Fig. 5a). These plants also had higher chlorophyll contents compared to dHo grown plants ($p < 0.05$), but lower values at day 8 compared to cHo. Nevertheless, on days 10 and 13, no differences in chlorophyll were observed between dHo + EC_{Fe} and cHo plants ($p > 0.05$). Results of cHo and cHo + BC (plants grown in complete nutrient solution supplemented with *N. oceanica* biomass harvested by centrifugation) showed no effects of *N. oceanica* on chlorophyll, and root and
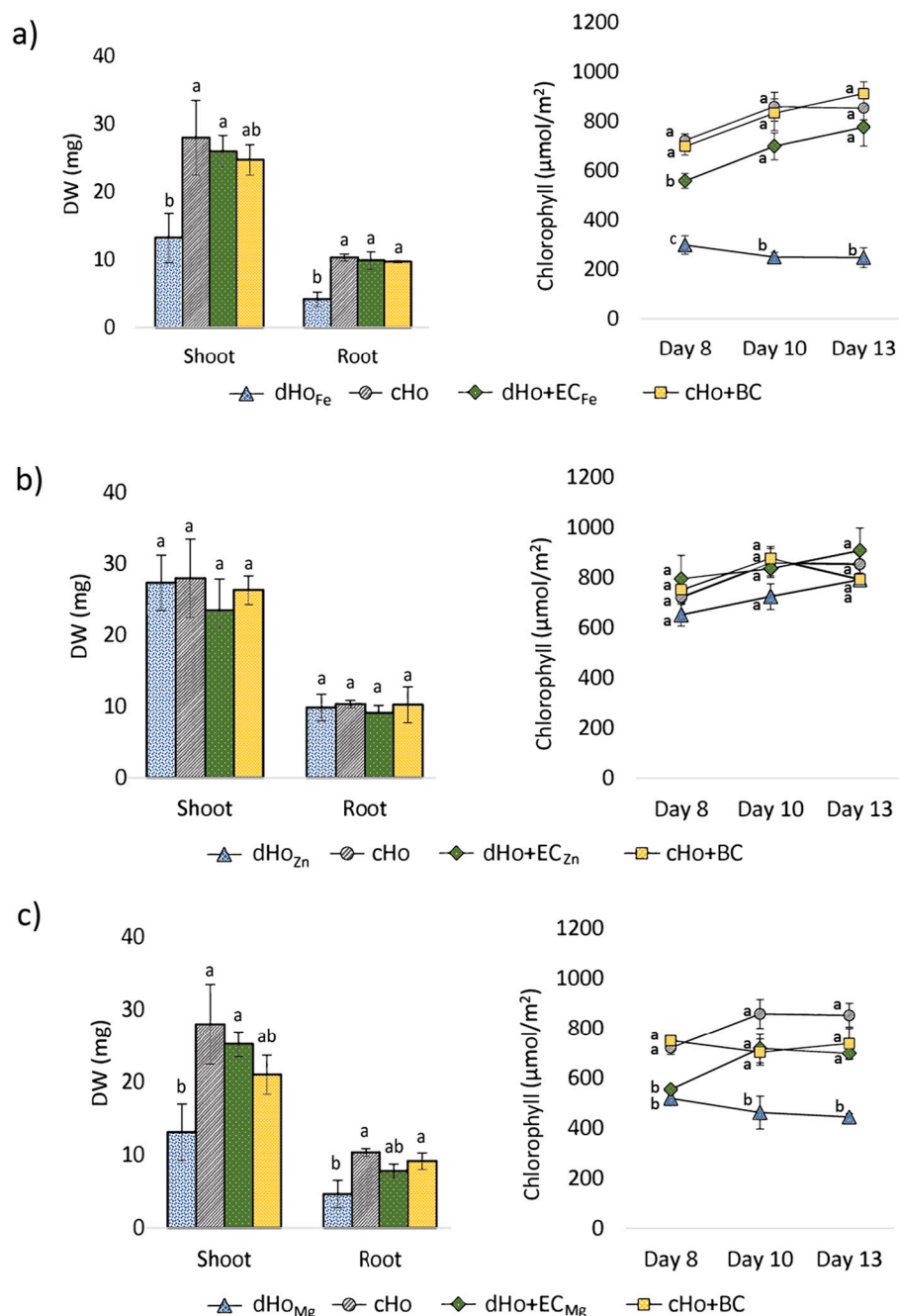

Fig. 4. Effects of biomass electrode metals (a) Fe, (b) Zn, and (c) Mg on *Solanum lycopersicum* shoot and root dry weight content (DW) and chlorophyll leaves content: dHo - Hoagland cultivation media deficient in the respective EC electrode metal; cHo- complete Hoagland cultivation media; dHo + $EC_{Fe/Zn/Mg}$ – deficient Hoagland supplemented with biomass harvested by electrocoagulation using Fe, Zn, or Mg electrodes, respectively; cHo + BC – complete Hoagland cultivation media with biomass harvested by centrifugation. Different letters indicate significant differences among Hoagland solutions (Tukey's test, $p < 0.05$). Data are shown as mean ± SD ($n = 3$).
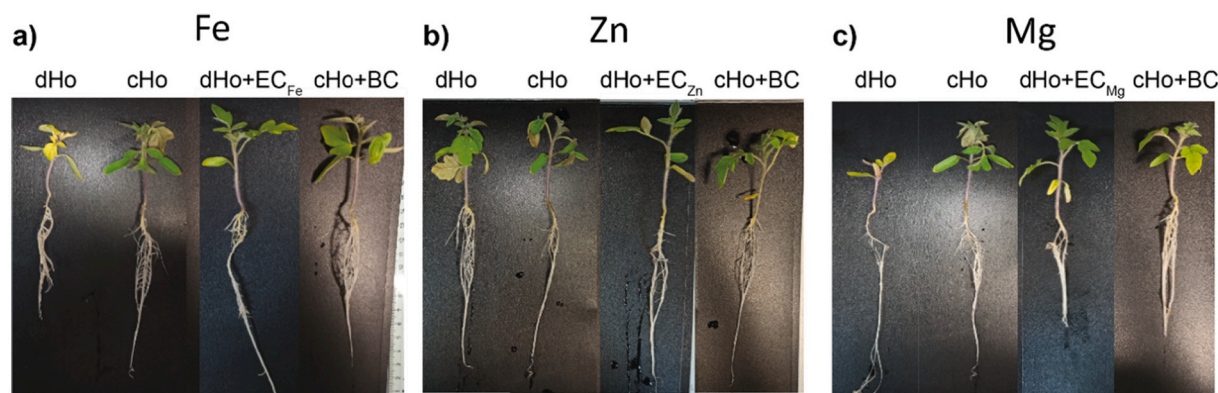
Fig. 5. Morphological differences of *Solanum lycopersicum* plants after 13 of cultivation days in different nutrient solutions to test Fe (a), Zn (b), and Mg (c) bioavailability. dHo - Hoagland cultivation media deficient in the respective EC electrode metal; cHo- complete Hoagland cultivation media; dHo + $EC_{Fe/Zn/Mg}$ − deficient Hoagland supplemented with biomass harvested by electrocoagulation using Fe, Zn, or Mg electrodes, respectively; cHo + BC – complete Hoagland cultivation media with biomass harvested by centrifugation.

shoot weights ($p > 0.05$).

Shoot and root weight from plants grown in Zn dHo, cHo, dHo + EC_{Zn}, and cHo + BC had no significant differences ($p > 0.05$; Fig. 4b; Fig. 5b). Also, no differences in chlorophyll content were found in plants of all treatments ($p > 0.05$). This suggests Zn original content in seeds was enough to avoid morphological and chlorophyll metabolic consequences. Zn is required in the plant at very low concentrations (Zn < 0.2 μM) but its deficiency is known to hinder the synthesis of antioxidant enzymes, which causes oxidative stress and leads to lipid or protein degradation (Pandey et al., 2012).

Plants grown in Mg dHo had lower shoot and root weight compared to plants grown in cHo ($p < 0.05$; Fig. 4c) with significant morphological effects (Fig. 5c). Also, lower chlorophyll contents were found between these groups, with differences increasing from 203 μmol/m^2 at day 8 to 396–406 μmol/m^2 at day 10 and 13. These significant harmful effects on plant development are expected due to Mg importance in photosynthesis by activating and regulating Rubisco binding to its carbamate group (Cakmak and Yazici, 2010; Portis, 2003). As a result, plants respond to Mg deficiency with a considerable reduction of CO_2 assimilation and consequent reduction in biomass formation (Hauer-Jákli and Tränkner, 2019), which occurred in this experiment.

Plants grown in dHo + EC_{Mg} had significantly heavier shoots than plants grown in dHo ($p < 0.05$), and similar weight compared to cHo plants ($p > 0.05$). However, no differences in the root weight were found between dHo + EC_{Mg} and dHo. Plants grown in dHo + EC_{Mg} solution had significantly higher chlorophyll contents compared to plants grown in dHo ($p < 0.05$) and no differences compared to cHo, except at day 8 where decreased chlorophyll contents were found. Similarly as Fe and Zn results, cHo and cHo + BC plants showed no differences in chlorophyll, and root and shoot weight ($p > 0.05$) at the same concentration of Mg EC biomass.

Higher chlorophyll content and growth observed in plants supplemented with Fe and Mg EC biomasses compared to dHo plants indicate that both Fe^{2+} and Mg^{2+} electrode metals were bioavailable to *S. lycopersicum*. In fact, no differences from cHo plants and no signs of phenotypic stress imply that these plants metabolized and transported EC metals to the active growing plant organs. As exception, partial Fe^{2+} and Mg^{2+} deficiencies were observed in these plants on day 8, due to lower chlorophyll than cHo plants. This impairment could be related to *N. oceanica* biomass sedimentation in the experimental tubes, which made microalgae-cell coagulated metals not available to the yet small roots at the first experimental days. Only at day 10, after root growth and transportation of Fe and Mg to the leaves, could plants recover from deficiency stress. Additionally, no symptoms of metal toxicity (chlorophyll or growth decreases) were found by supplying Fe, Zn, and Mg EC metals at 4 times higher concentrations than cHo. Also, no inhibitory or

growth-inducing effects were obtained from *N. oceanica* itself at 0.2, 5.8, and 338.1 mg/L concentrations (cHo + BC results).

Bioavailable Fe^{2+} and Mg^{2+} from EC could be a mineral boost in agricultural applications to prevent or solve stress related to these metals' deficiency, while providing plant beneficial effects due to microalgae growth-inducing properties (Ronga et al., 2019). This biomass could also be applied as an organo-mineral fertilizer to enrich agricultural soils with organic carbon, vitamins, phytohormones, Fe and Mg nutrients or supplied directly to the plant by foliar applications. The bioavailability of EC metals may substitute the need for synthetic chelates, which have serious environmental impacts (Pinto et al., 2014), while avoiding the need for natural degradable chelates, which increases fertilizer costs. Nevertheless, more studies are necessary to evaluate the percentage of bioavailable metals and their efficacy comparing to currently used chelates, as well as evaluating metal uptake by analysing plant metal concentrations. Still, the bioavailability of Zn electrodes needs to be proven, for example by extending Zn^{2+} stress duration.

3.5. Potential biomass applications

Nannochloropsis oceanica harvested using EC is enriched with minerals and potential applications for these biomasses have been suggested regarding their Fe, Zn, and Mg metal contents, such as food, feed, or agriculture (Figueiredo et al., 2022). However, potentially toxic elements found in this study, such as Pb, Cd, or As have to be considered, as these can compromise biomass applications according to legislation limits. Biomass from Zn and Mg electrodes had As contents in lower limits than animal feed thresholds but the same did not happen for Fe electrodes biomass (As >2 μg/g) considering a 12% moisture content (European Commission, 2013). However, Pb concentrations after EC surpasses the 10 μg/g threshold in animal feed products in all EC biomasses. As for foodstuffs, quantification limits have not been implemented for microalgae, though Pb in bivalve molluscs cannot exceed 1.5 μg/g (wet weight), inorganic As at 0.1–0.3 μg/g for rice products (wet weight), and Cd at 3 μg/g (dry weight) for food supplements, including seaweed dried products (Commission of the European Communities, 2006). For agricultural applications results showed Pb, As, and Cd concentrations are in accordance to the legislation of agricultural fertilizers in all EC biomasses (Regulation 2019/1009, 2019) but Cr was found at exceeding concentrations in biomass from Fe electrodes (>2 μg/g). Hence, strategies to avoid toxic metal contamination are necessary to fit targeted applications. For instance, the selection of purer Fe electrodes may solve the exposure risk of As and Pb for feed or food, while decreasing Cr contents for agricultural fertilizing products. Nevertheless, other mitigation strategies could be used, such as pH

control or the use of EDTA, as successfully reported by Figueiredo et al. (2022).

4. Conclusions

Electrocoagulation using Fe, Zn and Mg electrodes harvested and enriched *Nannochloropsis oceanica* biomass due to electrode corrosion and minerals precipitating from the culture media. The proven bioavailability of Fe and Mg electrode metals to *Solanum lycopersicum* plants make electrocoagulation a promising technology to harvest microalgae for agricultural applications. This biomass could be used as a plant growth inducer, while electrocoagulation metals could replace synthetic chelates by minimizing or solving plant nutrient stresses. Biomass toxic metal analyses revealed compromising contents of Pb and As for feed and Cr for agricultural applications but selecting purer electrodes could minimize As and Cr contents.

CRediT authorship contribution statement

Daniel Figueiredo: Investigation, Methodology, Data curation, Writing – original draft, Formal analysis. **Alice Ferreira:** Investigation, Methodology, Writing – review & editing. **Florinda Gama:** Investigation, Writing – review & editing, Validation, Resources. **Luisa Gouveia:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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