Biochemical Engineering Journal 232 (2026) 110216

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular article

How nutrients, pH and element concentration shape the biosorption of classical and emerging contaminants by living *Gracilaria gracilis*

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ARTICLE INFO

Keywords:
Biosorption
Rare Earth Elements
Complex mixtures
Nutrients
Response Surface Methodology

ABSTRACT

Marine and freshwater ecosystems are not only exposed to metals but to increasing macronutrient enrichment, causing environmental and health consequences. Macroalgae can bind contaminants and use nutrients in their growth. The influence of nutrients in the biosorption of Rare Earth Elements (REEs) and Classical contaminants, in complex mixtures, has not been fully covered. Response Surface Methodology (RSM) with Box-Behnken Design was applied to study the influence of biosorbent mass, element concentration and nutrient concentration on the removal response. Batch sorption experiments were performed for 48 h at salinity (10) and pH (6 and 8). Living biomass exhibited high removal rates (>80%) for REEs, Hg, and Pb, while As and Cd showed less affinity. Biosorbent dosage showed to be an influential parameter, while nutrients were not significant. Quadratic models exhibited the best fit after 48 h of exposure for all elements, except Hg. Despite the negligible effect of nutrients, in different optimisation scenarios, it was possible to improve the removal response in their presence. To better understand the environmental impact of adding nutrients or the need for high doses of biosorbent, LCA methodology would give further insights into the cost and environmental impacts of a macroalgae cultivation step.

1. Introduction

Human activities, such as industrial production and household consumption, have been responsible for the growing presence of metals and other contaminants in aquatic environments, which is also accompanied by an increased flux of macronutrients and nutrient-rich wastewater into water bodies [1,2]. Among different origins, the agricultural sector is one of the major contributors to the growing input of nitrate, while households and industry contribute most to phosphorus-associated pollution [3,4]. Other sources of nutrient enrichment are effluents from aquaculture and municipal and industrial discharges [5]. Nitrate and phosphate contribute, respectively, 20–50% and 35–55% to the eutrophication of water bodies [6].

Since marine and freshwater ecosystems and coastal organisms are not only exposed to metals but also to this growing macronutrient enrichment, nutrient removal during wastewater treatment is also needed to prevent the negative impact of nitrogen and phosphorus contamination. However, despite the phenomenon of eutrophication, phosphate (PO_4^{3-}) and nitrate (NO_3^-) are naturally present in seawater, being essential macronutrients not only for algae growth but also for other aquatic organisms, such as phytoplankton [2].

Potentially toxic elements (PTEs) are contaminants of particular concern due to their toxic nature, non-degradability, high persistence in the environment, bioaccumulation and bioamplification along the food chain, representing a potential public health concern [7]. Freshwater and rivers are the most exposed to contamination by PTEs arising from activities related to the leaching of pesticides and fertilisers from agricultural fields, acid mine drainage, discharges of untreated urban sewage, or industrial processing of fossil fuels [8,9]. Arsenic (As), Cadmium (Cd), Mercury (Hg), Lead (Pb), Niquel (Ni) and Chromium (Cr) are among this group of contaminants. Some of these elements receive particular attention due to their high frequency and persistence in aquatic ecosystems, as well as for their toxicity due to rapid assimilation and slower elimination resulting in bioaccumulation in the organism and magnification along the food chain [10,11]. Adding to these known classical contaminants, emerging contaminants such as Rare Earth

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https://doi.org/10.1016/j.bej.2026.110216
Received 25 January 2026; Received in revised form 2 April 2026; Accepted 18 April 2026
Available online 20 April 2026
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Elements (REEs) are increasingly being discharged into aquatic water bodies [12]. Electronic waste (E-waste) is a potential source of contamination when associated with low recycling rates and informal discharge procedures. These elements could be leached from E-waste, as has been reported for heavy metals, and subsequently enter the soils and reach groundwater [13]. REEs mining activities generate toxic waste, representing another source of leaching of these elements into ground and surface water [14,15].

Different methodologies have been exploited for the removal of contaminants from water, such as solvent extraction, ion exchange, electrolysis, reverse osmosis, membrane filtration or carbon adsorption [10,16,17]. For application in industrial or complex effluents, emerging solutions involving engineered nanoparticles [18], advanced drainage systems combined with adsorption processes [19] or ozone-based advanced oxidation techniques have been proposed [20].

As an alternative to these methodologies, biosorption has been gathering attention as a feasible, economical, simple and more environmentally friendly alternative for the remediation of contaminated waters from classic and emergent pollutants [21,22]. Macroalgae have been reported to remove classic contaminants, such as metals (Hg, Cd, Pb), and emergent contaminants, such as REEs [12,22,23]. Different studies have been addressing the biosorption of metals by macroalgae in the context of complex and multi-element mixtures [24-27], encompassing the interactions between metals and moving closer to mimicking real effluents. However, the effect of the mixture of these elements on the biosorption ability of macroalgae, in combination with nutrients such as nitrate and phosphate, has not yet been addressed in the literature. The influence of these common nutrients, often associated with eutrophication, on the simultaneous removal of rare earth elements (REEs) and classical contaminants remains unexplored. This study aims to fill this gap by examining how their presence in complex mixtures affects biosorption in macroalgae, providing insights into the environmental implications of increasing nutrient and emerging contaminant inputs into aquatic ecosystems. To the best of our knowledge, no studies have specifically investigated the effect of nutrients on the biosorption of REEs in complex mixtures of classical and emerging contaminants in macroalgae.

The present work evaluates the biosorption capacity of living macroalgae under varying operational conditions (pH, initial contaminant concentration, and biosorbent mass) as well as different contamination scenarios (e.g., the presence of nutrients), aiming to reproduce the diversity of chemical species and physicochemical parameters characteristic of industrial effluents. To model these influences, Response Surface Methodology (RSM) combined with Box-Behnken Design was applied.

2. Material and methods

2.1. Biosorbent preparation

2.1.1. Macroalgae collection

Gracilaria gracilis was collected at the Aveiro estuary (Portugal, 40º 34'35.1 N, 8º45'08.1 W) [12] and transported to the laboratory in isothermal plastic bags, containing estuary water. Before exposure to the contaminants, the macroalgae were rinsed with tap water to remove debris and epibionts from the collection site. Samples of the biomass collected were immediately freeze-dried for quantification of the inherent metal concentrations (baseline). Basal concentrations of the macroalgae's biomass are displayed in Table S1.

Macroalgae were maintained in glass aquariums equipped with air pumps and filled with filtered seawater until the beginning of the experiments. The aquariums were kept under natural light with a photoperiod of 12 h, at room temperature (20 ± 2 ºC), without any nutrient supply. To prepare the biomass for exposure to contaminants, several pieces were cut from the macroalgae thallus, with similar sizes between 3 and 5 cm.

2.2. Reagents

All laboratory material was washed and filled with 25% (v/v) HNO_3 solution (Merck, supra-pure, 65% m/m) for at least 24 h and then rinsed with ultrapure water (Milli-Q, 18 MΩ/cm). Mono-element solutions of REEs in HNO_3 (1–7%) solutions were obtained from certified suppliers: Alfa Aesar (Y, Dy), Inorganic Ventures (Nd), Plasma Cal (La, Eu), and Sigma Aldrich (Gd). The certified standard solutions of mercury nitrate ($Hg(HNO_3)_2$: 1001 ± 2 mg L^{-1}), arsenic acid (H_3AsO_4: 1000 ± 2 mg L^{-1}), cadmium nitrate ($Cd(HNO_3)_2$: 1000 ± 2 mg L^{-1}), and lead nitrate ($Pb(HNO_3)_2$: 1000 ± 2 mg L^{-1}) were purchased from Merck. The preparation of the nutrient solution was achieved by the dissolution in distilled water of commercial solid salts of KH_2PO_4 and NO_3 purchased from Merck and Sigma-Aldrich, stored in the fridge until further use.

2.3. Seawater collection and preparation of contaminated saline solutions

Seawater used to prepare the contaminated solutions for macroalgae exposure was collected at Barra beach (Portugal, 40°33′N, 8°46′W). After transportation to the laboratory, seawater was filtered through 0.45 µm Millipore membranes and kept in the dark at 4 °C until further use. A physicochemical characterisation of the seawater, including pH, conductivity, salinity, and major and minor elements analysis, was already reported by [24].

The contaminated saline solutions to which the living macroalgae were exposed were prepared by dilution of the filtered seawater with ultrapure water to a final salinity of 10. This salinity has been chosen since it is a common value in estuaries that are receptacles of industrial discharges containing the pollutants present in the mixture [24]. Adequate volumes of REEs standard commercial solutions were added to spike the saline water, ensuring equimolar concentrations of the elements for a more accurate comparison. The real concentrations of these elements in the prepared mixtures were between 75 and 1036 µg L^{-1} and were selected considering reported values for contaminated waters [28].

2.4. Quantification of elements in solution

Element concentrations in the saline solution were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) on a Thermo Scientific X Series Quadrupole instrument. Calibration curves were built with 5 multi-element standards, previously diluted from a certified standard in HNO_3 1%, with concentrations ranging from 0.1 to 10 µg L^{-1}. The limits of quantification were 2 µg L^{-1} for As, 0.1 µg L^{-1} for Cd, 0.1 µg L^{-1} for Pb, 0.2 µg L^{-1} for Y, 0.02 µg L^{-1} for La, Nd and 0.01 µg L^{-1} for Eu, Gd, and Dy, with an acceptable relative standard deviation among replicates (≤10%). The Limit of Quantification (LOQ) was assumed to be the concentration of the lowest standard used in the calibration curve, while 1/3 of this value was assumed to correspond to the Limit of Detection (LOD) [29]. To avoid matrix interferences, all samples were diluted 10 times with HNO_3 2%, before quantification [24]. Mercury quantification in water samples was performed by cold vapour atomic fluorescence spectroscopy (CV-AFS) in a PSA 10.025 Millennium Merlin Hg analyser and using $SnCl_2$ (2% m/v in HCl 10% v/v) as a reducing agent (Henriques et al., 2019). In the range of operational conditions used, the LOQ was 0.021 µg L^{-1} (n = 20), with an acceptable relative standard deviation among replicates (≤5%). Moreover, each sample was analysed at least 3 times with an acceptable relative standard deviation among replicates of ≤ 5% for Hg and ≤ 10% for the other elements under study.

2.5. Experimental design

2.5.1. Biosorption assays

Box-Behnken Design (BBD) of 3 factors - 3 levels with equidistant values (− 1; 0; +1) was applied to generate a matrix of the studied

condition's combination to be experimentally performed [30,31]. The variable parameters evaluated by the model were the initial concentration of the contaminants in equimolar concentrations (1, 3 and 5 μM), mass of the sorbent (2, 8 and 14 g, FW), and concentration of nutrients (0 μg P/0 μg N; 100 μg P/ 500 μg N; 200 μg P /1000 μg N). The experimental matrix conditions are shown in Table 1. Central point conditions were run in triplicate. The central point conditions represent the intermediate levels (0,0,0) for the evaluated parameters – biosorbent mass (8 g), initial contaminant concentration (3 μM) and nutrient concentration (100 μM of P /500 μM of N). The mixture to which the macroalgae biosorbents were exposed was composed of 10 elements: As, Cd, Y, La, Eu, Gd, Nd, Dy, Pb and Hg. These elements were chosen to mimic the complexity of an industrial effluent, which has both classical contaminants and emerging contaminants. In each condition, all metals are in equimolar concentrations. Batch sorption experiments were performed in Schott Duran® glass flasks (1 L), at room temperature (20 ± 2 °C), by contacting fresh living macroalgae with 1 L of saline solutions spiked with the metals under study at salinity 10 and pH 6. After cutting the macroalgae, fresh weights of approximately 2, 8, and 14 g were used according to each condition, and the corresponding mass was added to flasks containing the contaminant mixtures. No further pretreatment was applied before exposure. Following addition to contaminated seawater, solution samples (ca. 10 mL) were collected at pre-defined times up to 48 h of exposure (1, 3, 6, 24, 48 h contact time) and then acidified to pH < 2 with HNO_3 (65% m/m) for further analysis. Quality control was assured by running four control assays with a combination of the boundary conditions of the initial concentration of contaminants and nutrient, without the addition of biomass, at salinity 10 and pH at 6 (1 μM with 0 μg of P and N; 5 μM with 200/1000 μg of P and N; 1 μM with 200/1000 μg of P and N and 5 μM with 0 μg of P and N). Blanks, defined as macroalgae with only saline water without contaminants, were also run together.

2.5.2. Stability of contaminant mixture at different pH values

Before initiating the BBD experiments at pH 6, control assays (n = 2) were conducted to assess the stability of the elements in solution at different pH values. These controls included two contaminant concentrations (1 and 5 μM) of the same mixture of elements (As, Cd, Y, La, Eu, Gd, Nd, Dy, Pb and Hg), at salinity 10 and different pH values (6.0, 7.6, 7.8, 8.0, and 8.2), without the presence of macroalgae. Assays were performed in Schott Duran® glass flasks (1 L), at room temperature (20 ± 2 °C). Solution samples (ca. 10 mL) were collected at pre-defined times of assay until 168 h of exposure (6, 24, 48, 72, 168 h contact time) and then acidified to pH < 2 with HNO_3 (65% m/m) for further analysis.

2.6. Data analysis

The removal efficiency (*R*, %) of the chemical elements was calculated based on their initial concentration (C_0, μg L^{-1}) and concentration at the time *t* (C_t, μg L^{-1}) in the solution by using the following equation (Henriques, 2017b):

$$R = \frac{(C_0 - C_t)}{C_0} \times 100 \quad (1)$$

Design-Expert software (v13.0.1.0) was applied to obtain the surface response analyses, as well as for the graphical treatment of data. For the response surface analysis, the second-order polynomial model was used, following the equation:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i<j}^{k} \beta_{ij} X_i X_j \quad (7)$$

where Y is the predicted response value; β_0 is a constant; β_i is the linear coefficient, β_{ii} is the quadratic coefficient; βij is the interaction coefficient; and X are the coded independent variables. Analysis of variance (ANOVA) was applied to determine the factors' significance as well as the interactions between them using Fisher's test and its associated probability p (F). The fitness of the polynomial model equations was evaluated by the coefficient of determination, R^2, and the adjusted coefficient of determination, R^2_{adj}. Regression analysis and 3-D response surface plots helped to estimate the optimal conditions.

3. Results

3.1. Kinetic profiles of elements in the absence of macroalgae at pH between 6 and 8.2

With the aim of understanding which fraction of the elements in solution is available for binding to the biosorbent, their behaviour without the presence of the macroalgae was first studied for different combinations of pH and contaminant concentrations. Fig. 1 illustrates the behaviour of As, Y, La and Dy during 168 h, in a complex mixture at two different concentrations (1 and 5 μM) and pH values of 6, 7.6, 7.8, 8.0 and 8.2. It is possible to observe that, independent of the concentration or element, at pH 6, no alterations in the normalised concentration are registered during 168 h. However, with the gradual increase of pH, a different effect is observed for the two concentrations. As an example, while the normalised concentration of As shows no significant alterations, for all the tested pH levels at 1 μM, it decreases significantly at 5 μM (almost 80%) within the first 24 h. At 1 μM, the highest decreases occur within 72 h. Until pH 7.8, generally the concentration decreases in 20% or less, while beyond pH 8 the losses can reach > 40%.

Table 1
Design matrix of each experimental trial condition (Factor A – biosorbent mass (g); Factor B contaminant concentration (μM); Factor C - nutrient concentration (P/N (μM)).

Coded level of variables				Real values of variables		
Experiment	**A**	**B**	**C**	**Biosorbent mass (g)**	**Contaminant concentration(μM)**	**Nutrient concentration (μM)**
1	-1	+ 1	0	2	5	100/500
2	+ 1	+ 1	0	14	5	100/500
3	+ 1	0	+ 1	14	3	200/1000
4	0	0	0	8	3	100/500
5	0	0	0	8	3	100/500
6	0	+ 1	-1	8	5	0/0
7	+ 1	-1	0	14	1	100/500
8	0	0	0	8	3	100/500
9	-1	0	+ 1	2	3	200/1000
10	+ 1	+ 1	+ 1	8	5	200/1000
11	-1	0	-1	2	3	0/0
12	+ 1	-1	+ 1	8	1	200/1000
13	+ 1	0	-1	14	3	0/0
14	0	-1	-1	8	1	0/0
15	-1	-1	0	2	1	100/500

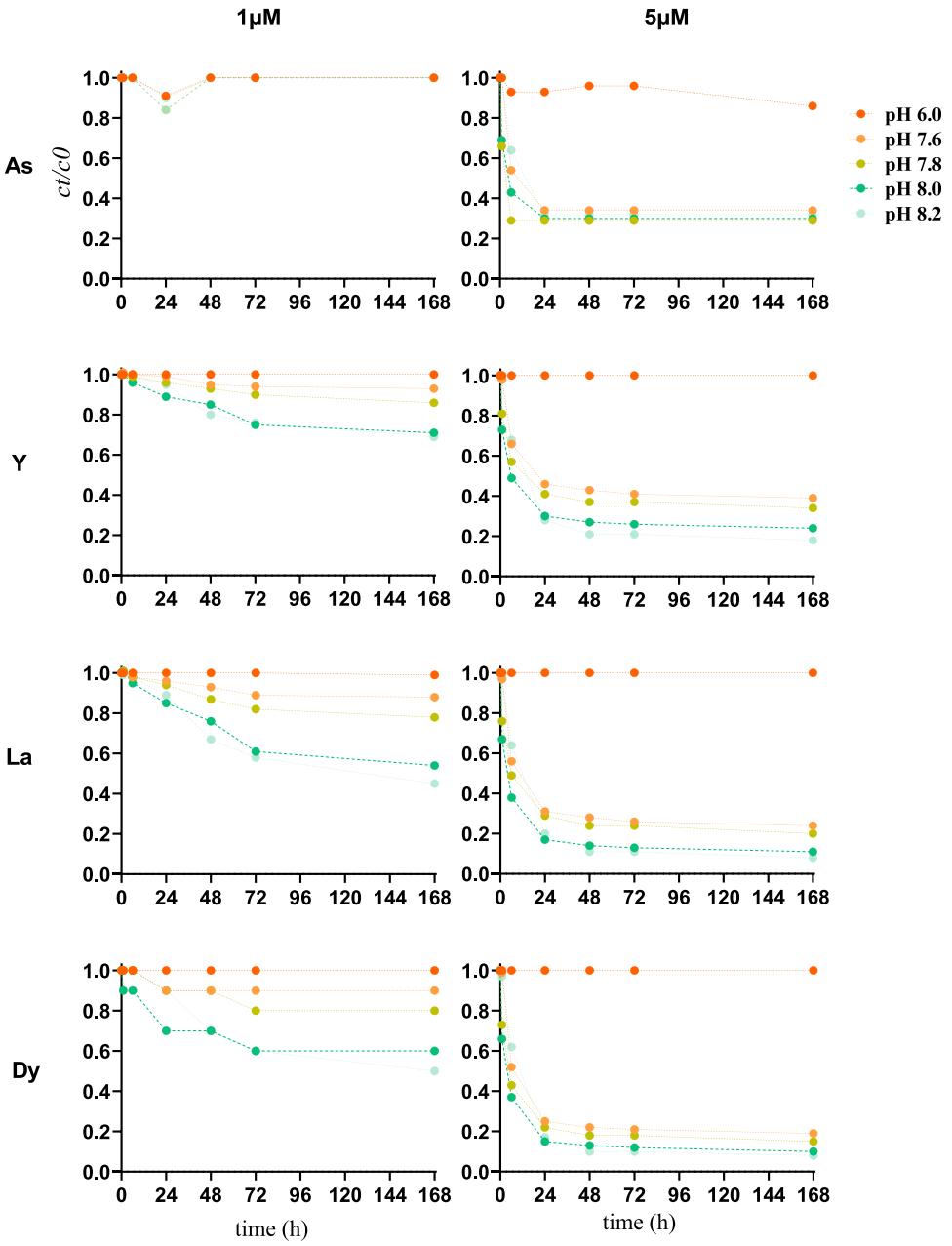


Fig. 1. – Normalised concentrations (n = 2) in water during 168 h for As, Y, La and Dy at different pH, at concentrations of 1 µM (left) and 5 µM (right).

This pattern repeats for all the elements shown and for the other REEs present in the mixture, except for the elements Hg and Cd, which do not suffer any alteration at any concentration or pH tested. While there are no observable variations in the concentration of all elements at pH 6, for pH 8 (data provided in Figure S1), it is possible to see a decrease in the concentration of the elements even in the absence of the macroalgae, except Hg and Cd, which are stable in solution. However, the accentuated decrease occurs after 6 h, reaching a plateau of equilibrium.

3.2. *Kinetic profiles of elements for central point conditions*

Fig. 2 presents the removal kinetics profiles for Cd, Y, Gd and Pb when exposed to the living macroalgae *G. gracilis* at pH 6, at the conditions of 8 g of sorbent exposed to a mixture of As, Cd, Y, La, Eu, Gd, Nd, Dy, Pb and Hg in equimolar initial concentrations (3 µM), in the presence of 100 µg of P and 500 µg of N, for 48 h of exposure (n = 3). From the studied range, pH 6 was selected for further studies since the concentration of all elements remains stable, allowing to evaluate the sorption ability of the macroalgae at higher concentrations. Also represented in the figure are the controls that were run together with the experiment, describing the behaviour of the elements at the same pH and concentration, but without the presence of the macroalgae, in two different conditions, which are the presence or the absence of nutrients.

Cadmium and As removals were globally low, while the REEs, represented by Y and Gd, achieved removal rates greater than 80% after 48 h of exposure. Lead and Hg were removed from the solutions more intensively than Cd and As, with a kinetic profile more similar to those observed for the REEs. The kinetic profiles show that the most significant part of the removal occurs within 6 h of exposure, almost reaching a plateau at 24 h and finally an equilibrium state, after 48 h. The replicas were consistent with each other, with standard deviations between 0.02 and 0.08. Therefore, the removal percentages herein obtained due to their reproducibility were further analysed with the help of the methodology of response surface (RSM).

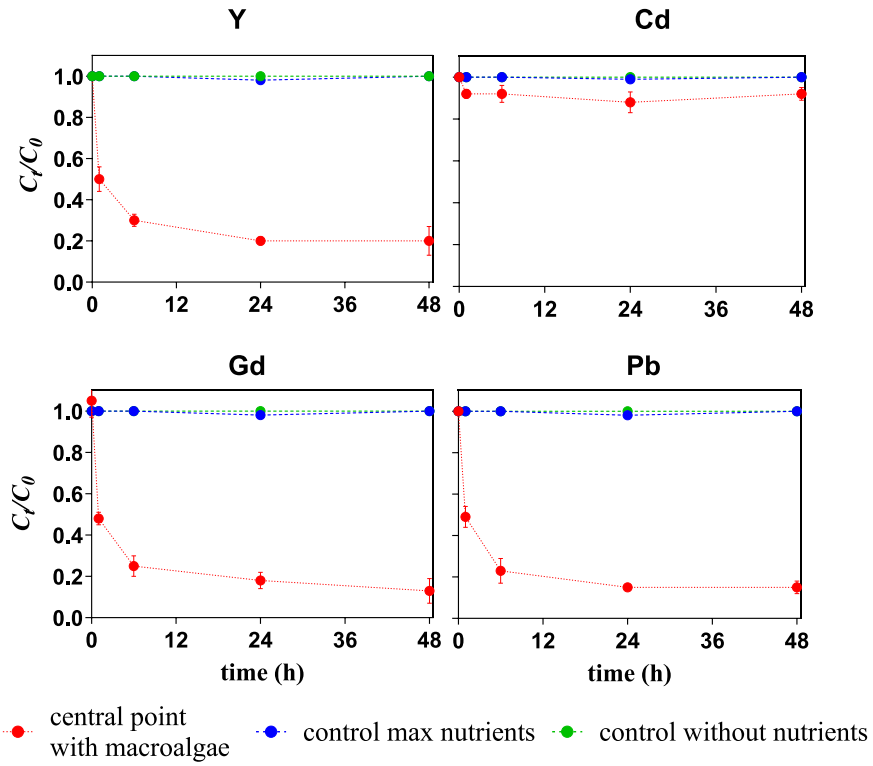


Fig. 2. – Normalized removal profiles for the elements Y, Cd, Gd and Pb during 48 h at central point conditions from assays at pH 6; the red line represents the removal profile for each element at central point conditions (n = 3) (STD between 0.02 and 0.08) of 8 g of sorbent exposed to a mixture of As, Cd, Y, La, Eu, Gd, Nd, Dy, Pb and Hg in equimolar initial concentrations (3 μM), in the presence of 100 μg of P and 500 μg of N; the blue and green lines, respectively, represent the normalized concentrations for each element without the presence of macroalgae (control) combined with the maximum concentration of nutrients added (200 g of P and 1000 μg of N) and without the addition of nutrients.

3.3. Removal percentages for all elements at 6, 24 and 48 h of exposure

Table 2, **S2** and **S3,** respectively, present the obtained removal percentages for 6, 24 and 48 h of exposure for all the elements, at pH 6. Focusing first on the element Hg, the highest removal (%) by *Gracilaria gracilis*, achieved at 6 h, 24 h and 48 h, respectively, was 91.7, 96.0 and 96.4%, on experimental assays 2 and 14 with the operational parameters of initial concentration of 5 μM; biomass dosage of 14 g L^{-1} and concentration of nutrients P and N of 100 and 500 μM; initial concentration of 1 μg L^{-1}; biomass dosage of 8 g L^{-1} and concentration of nutrients P and N of 0 μg. After 6 h of exposure, removal percentages varied from 31.2% to 91.7%, while after 24 h, from 33.8% to 96.0% and at 48 h of exposure, from 34.0% to 96.4%. The lowest removal performance was observed at 6 h of exposure with 31.2% of Hg removed for experimental assay 11 (initial concentration of 3 μg L^{-1}; biomass dosage of 2 g L^{-1} and 0 μM of concentration of nutrients P and N). For the elements Cd and As, after 48 h, removal percentages were between 3.3% and 54.9% and 0 and 38.6%, respectively. Focusing now on the element Pb, the removal percentages obtained after 48 h of exposure were between 39.3% and 94.7%. For the Y, removals ranged between 35.0% and 84.0%, La between 42.3% and 93.3% %, for Nd between 64.6% and 93.3%, for Eu between 66.4% and 92.9%, for Gd between 65.4% and 94.6%, for Dy between 41.3% and 87.1%

Table 2
– Removal percentages (%) of all contaminants for each experimental assay after 6 h of exposure at pH 6.

Experimental assay	**Assay BBD-B (pH6) Removal (%) - 6 h**									
Elements	**As**	**Y**	**Cd**	**La**	**Nd**	**Eu**	**Gd**	**Dy**	**Pb**	**Hg**
1	11.0	9.5	4.6	22.4	32.7	37.0	34.3	28.4	17.5	48.0
2	24.2	73.4	17.3	77.0	76.9	75.8	78.6	74.6	84.9	91.7
3	26.1	74.7	19.8	79.7	78.1	76.2	78.5	74.4	87.2	62.7
4	8.1	68.3	4.2	80.6	81.3	77.8	76.5	74.2	63.3	87.0
5	13.7	72.8	8.4	77.7	79.4	78.4	78.5	77.1	88.1	70.9
6	17.9	47.0	9.8	50.7	62.5	67.7	65.5	61.4	70.5	69.3
7	0	72.1	49.9	71.8	61.9	60.3	67.6	62.0	86.0	91.5
8	0	54.3	4.6	59.0	64.6	64.1	63.3	60.5	51.9	48.1
9	3.4	19.1	7.7	29.7	34.2	36.8	37.3	30.4	20.7	45.6
10	13.9	45.2	7.5	49.0	55.4	58.0	33.2	55.5	57.6	65.4
11	0	16.8	6.0	27.4	35.7	38.4	36.5	31.8	20.2	31.2
12	0	60.9	43.4	54.6	47.6	41.6	82.3	46.5	85.3	78.5
13	29.2	83.5	31.3	87.3	87.4	83.7	84.9	81.2	91.9	85.1
14	0	71.2	52.7	80.9	81.7	81.9	94.6	79.2	85.6	81.0
15	0	44.6	8.5	47.9	49.6	44.7	83.8	41.3	42.5	53.9

3.4. Model equations and fitting parameters

Table 3 presents the reduced equations with the significant terms affecting the evaluated response (removal %) for the elements Y, La, Nd, Eu, Gd, Dy, Pb and Hg, for 6 and 48 h of exposure. Reduced models and the respective equations were used since better fits were achieved when the terms that were not significant were removed from the equations. For each selected element and at two exposure times (6 and 48 h), the model that best fits the experimental data is presented, along with the corresponding R^2, R^2_{adj} and p-value. Globally, the reduced quadratic model provided the best fit for all the elements at 48 h except for Hg, which was better described by a linear model. When observing the evolution of the surface responses from 6 h to 48 h, some elements maintain the same model fit, such as Y, Dy and Hg, while others move from Linear to Quadratic fit. The R^2 for 6 h were between 0.6163 and 0.9811, and the R^2_{adj} was between 0.5116 and 0.8475. The values of these same parameters at 48 h vary between 0.7114 and 0.9652 for R^2, while between 0.6327 and 0.9391 for R^2_{adj}. These values are indicative of the better goodness of fit and accuracy between the experimental data and the calculated data for the responses at 48 h of exposure. A poorer correlation was obtained for the element Hg, for the two exposure times, when compared to the other elements, which present good adjustments, corroborated by the values of R^2 and R^2_{adj} obtained, which are indicative of the robustness of the models. Moreover, the obtained F-values (9.04–37.0) and p-values ($p < 0.05$) support the significance of the models obtained. Focusing at 48 h of exposure (when A, B and C represent, respectively, the factors biosorbent mass, element concentration and nutrient concentration), for the REE, p-values < 0.0500 indicated that Mass, (Mass×element concentration), and Mass² were the most significant terms, while for the representative of the classical elements herein, Hg and Pb, were Mass, Mass² and element concentration. Regarding the influence of the significant terms, the linear variable of biosorbent Mass is positive for all elements presented (REEs, Hg and Pb), indicating that higher doses of biosorbent result in higher removal responses. For the quadratic variable of biosorbent Mass², the opposite is found, since for the REEs presented, plus Pb, it presents a negative value. The interpretation of this can be associated with the existence of a maximum optimum value for the biosorbent dose, after which it starts to have an opposite effect on the removal response. Regarding the linear interaction between biosorbent Mass and Element concentration, it has a positive effect on all the REEs presented. The linear variable of Element concentration presents a negative effect for Hg, while for some REEs it has a positive value, for example, for Nd, Eu and Dy, being not significant for Pb. The quadratic variable of Element concentration presents a negative impact for the elements La, Nd, Eu and Dy, indicating the shape of a curvature and a maximum optimum value. Regarding the linear variable of nutrient concentration, it showed not to be significant for any of the presented elements of the mixture, except Cd (p-value 0.0326), where it had a negative effect on the response.

3.5. Response surface for chosen elements and variables affecting the removal of contaminants

Figs. 3 and 4 present the 3D response surface plots for the different elements of the mixture after 48 h of exposure, highlighting the influence of the studied variables on the removal response evaluated. These three-dimensional representations of the reduced quadratic models presented (Table 3) more easily illustrate the effects of the significant variables Mass, Element concentration, (Mass×element concentration) Mass², Element concentration² for the representatives of the classical elements and REEs herein (Y, Nd, Pb and Hg). For the element Pb (Fig. 3 – C) it can be observed that an increase of the element concentration from 1 to 5 μM shows not to affect significantly the removal efficiency (40%), while in the case of Hg (Fig. 3 – D) the same increase in the concentration results in the decrease of the removal efficiency from 90.0% to 69.8% (for the intermediate value of biosorbent mass and nutrient concentration). As for the variable of biosorbent mass, an increase from 2 g/L to 14 g/L results in an increase from 44.9% to 90.1% for the element Pb, while for Hg, the increase is from 59.7% to 100%. For the elements Y(Fig. 3 – A) and Nd (Fig. 3 – B), the same increase in the biosorbent mass results in lower variations in the response, from 55.2% to 82.0% for Y and from 75.3% to 93.5% for Nd. As for the

Table 3
– Fitting parameters for response surface models better adjusted to experimental data on the removal of each contaminant (Y, La, Nd, Eu, Gd, Dy, Pb and Hg) by living macroalgae *Gracilaria gracilis*, at pH 6 and reduced equations with the significant terms affecting the evaluated response (removal %) for 6 and 48 h of exposure to living macroalgae *Gracilaria gracilis*.

Living macroalgae – *Gracilaria gracilis*								
	6 h				48 h			
	p-value	**R^2**	**R^2_{adj}**	**Reduced equation**	**p-value**	**R^2**	**R^2_{adj}**	**Reduced equation**
Y	< 0.0001	0.8911	0.8475	R (%) = 60.0 + 26.7 Mass - 9.21 element concentration – 2.33 nutrients concentration −10.7 Mass²	0.0035	0.8700	0.7977	R (%) = 76.9 + 13.4 Mass - 4.14 element concentration + 1.47 nutrients concentration + 9.85 (Mass × element concentration) - 8.35 Mass²
La	0.0009	0.7628	0.6982	R (%) = 59.7 + 23.6 Mass - 7.01 element concentration – 4.16 nutrients concentration	0.0001	0.9441	0.9022	R (%) = 89.8 + 15.3 Mass - 2.16 element concentration + 1.42 nutrients concentration + 10.1 (Mass × element concentration) - 12.9Mass² – 5.91 element concentration²
Nd	0.0063	0.6604	0.5677	R (%) = 61.9 + 19.0 Mass - 1.66 element concentration – 6.50 nutrients concentration	< 0.0001	0.9652	0.9391	R (%) = 92.0 + 9.10 Mass + 2.04 element concentration – 0.84 nutrients concentration + 4.70 (Mass × element concentration) - 7.59 Mass² – 7.12 element concentration²
Eu	0.0066	0.6574	0.5640	R (%) = 61.5 + 17.4 Mass + 1.25 element concentration – 7.39 nutrients concentration	< 0.0001	0.9584	0.9272	R (%) = 89.5 + 8.24 Mass + 2.23 element concentration – 0.99 nutrients concentration - 6.25 Mass² – 7.23 element concentration²
Gd	0.0126	0.6124	0.5067	R (%) = 66.4 + 14.7 Mass - 14.6 element concentration – 6.28 nutrients concentration	0.0005	0.8853	0.8216	R (%) = 87.0 + 6.15 Mass - 6.93 element concentration - 1.25 nutrients concentration + 6.58 (Mass × element concentration) −5.09 Mass²
Dy	0.0011	0.8164	0.7430	R (%) = 64.9 + 20.4 Mass - 1.14 element concentration – 5.85 nutrients concentration −11.9 Mass²	0.0005	0.8883	0.8262	R (%) = 85.2 + 12.2 Mass + 2.70 element concentration – 0.08 nutrients concentration −10.0 Mass² – 8.47 93 element concentration²
Pb	0.0003	0.8078	0.7554	R (%) = 63.6 + 31.1 Mass - 8.61 element concentration – 2.17 nutrients concentration	< 0.0001	0.9076	0.8707	R (%) = 84.3 + 23.1 Mass - 1.2 (Mass × element concentration) – 0.41 nutrients concentration - 16.3 Mass²
Hg	0.0119	0.6163	0.5116	R (%) = 67.3 + 19.0 Mass - 3.81 element concentration −1.80 nutrients concentration	0.0026	0.7114	0.6327	R (%) = 79.9 + 20.2 Mass - 10.1 element concentration – 0.33 nutrients concentration

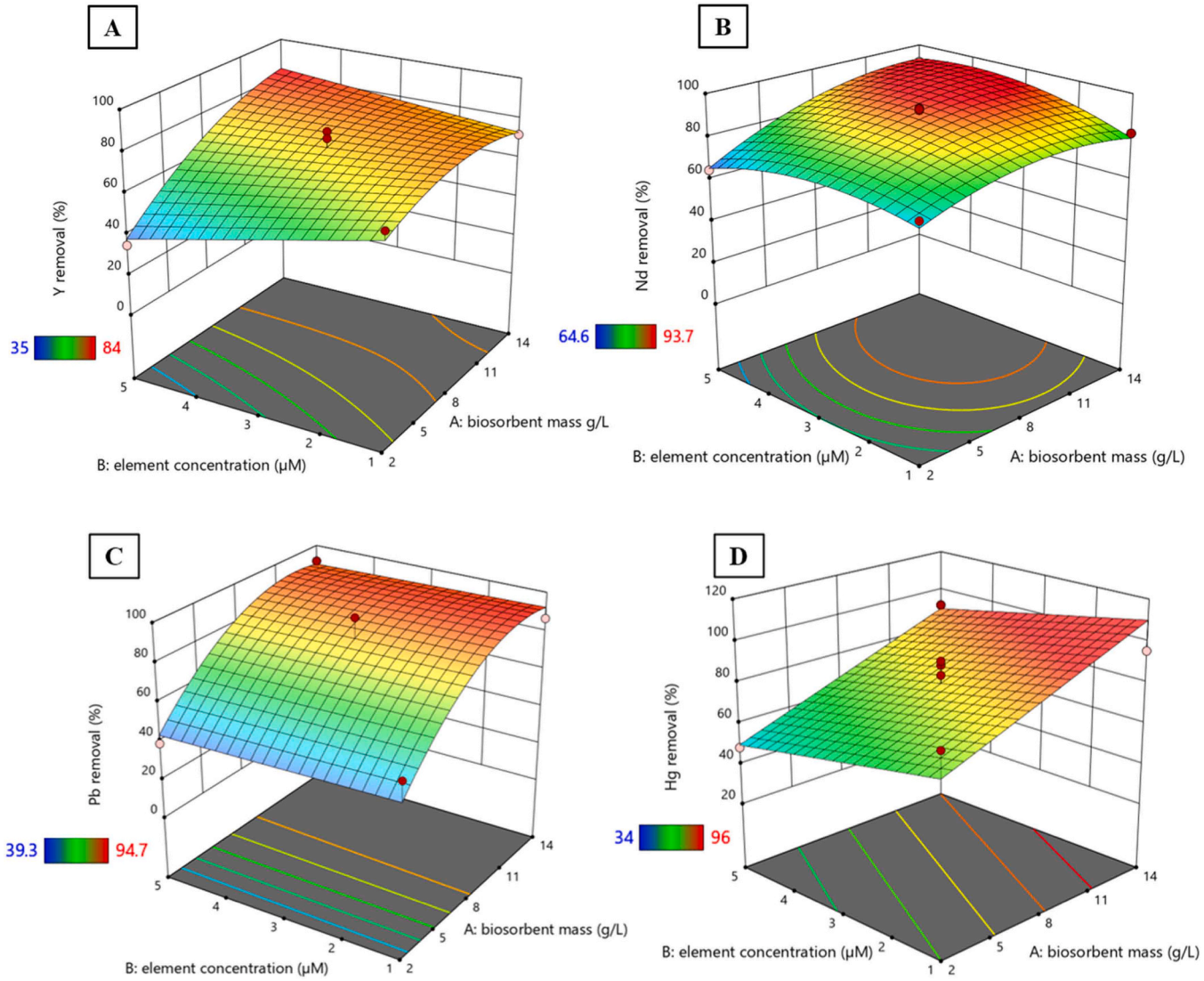


Fig. 3. – Response surface (pH 6) for the removal percentages obtained by *Gracilaria gracilis* after 48 h of exposure for Y, Nd, Pb and Hg in a mixture of As, Cd, Y, La, Eu, Gd, Nd, Dy, Pb and Hg in equimolar initial concentrations (1,3 and 5 μM), in the presence of different concentrations of P and N nutrients and different doses of sorbent, 2 g, 8 g and 14 g; A axis is fixed at the factor of biosorbent's dosage and axis B is fixed at contaminants concentration; value of nutrients is for model – 100 μg and 500 μg of P.

influence of element concentration, the increase from 1 μM to 5 μM results in a variation of 81.0–72.8% and 82.8–86.9% in the removal responses of the two elements, respectively. Focusing now on the combined influence of nutrients concentration and the other variables of biosorbent mass and element concentration for the elements Hg (Fig. 4 – A and B) and Eu (Fig. 4 – C and D), for Hg, independently of the concentration of nutrients, there is an increase in the removal efficiency when the biosorbent mass increases from 2 g/L to 14 g/L and a decrease of the efficiency when the element concentration varies from 1 μM to 5 μM. For the element Eu, the same increase in the biosorbent mass results in a lower variation of the response, while there is an optimum value of element concentration for the highest response, also independently of the influence of the nutrient concentration.

3.6. Optimal conditions for removal at 48 h of exposure

Optimal parameters of operation for the variables biosorbent mass, element concentration and nutrient concentration were obtained in Design-Expert by non-linear regression and are displayed in Table 4, as well as the expected removals in the different scenarios. Optimization was carried in three different scenarios (1, 2 and 3): - In scenario 1, the optimization was achieved in the presence of the maximum nutrient concentration (factor C) with the other variables A (biosorbent mass) and B (element concentration) set at any value, while in scenario 2 the same was followed, but without the presence of nutrients (Factor C was in minimum level); in scenario 3, the target was to minimize the amount of biosorbent used (factor A), while the other two variables were set at any value.

4. Discussion

4.1. New insights into the biosorption of complex mixtures by living macroalgae

The effect of parameters such as initial ion concentration, contact time and biosorbent dosage has been exploited through the methodology of response surface design [32,33]. Combined with the design of experiments, the methodology has been used in studies to optimise the biosorption with different biosorbents and metals, such as the removal of Co from synthetic wastewater by the brown macroalga *Padina*

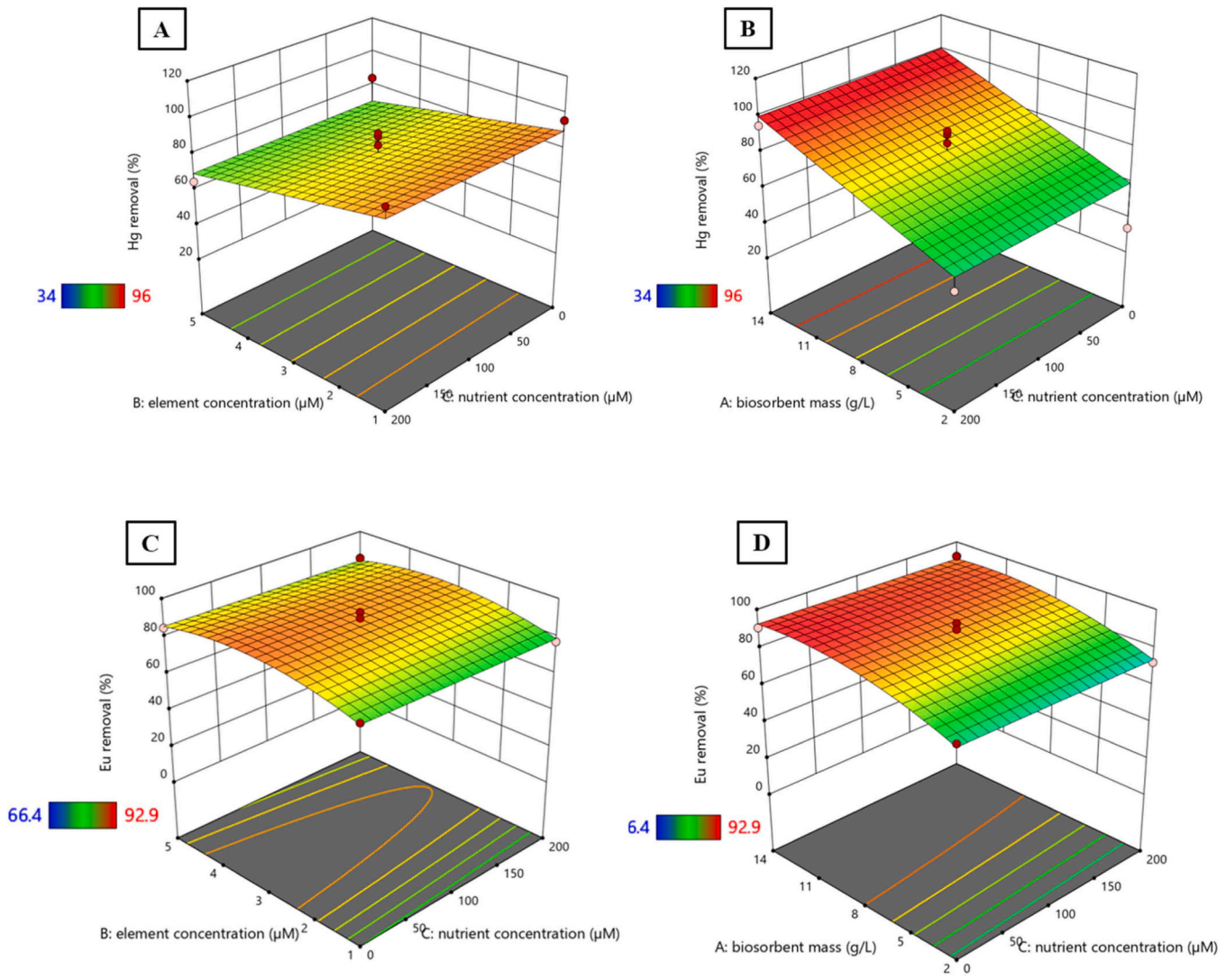


Fig. 4. – Response surface (pH 6) for the removal percentages obtained by *Gracilaria gracilis* after 48 h of exposure for Hg and Eu, in a mixture of As, Cd, Y, La, Eu, Gd, Nd, Dy, Pb and Hg in equimolar initial concentrations (1,3 and 5 μM), in the presence of different concentrations of P and N nutrients and different doses of sorbent, 2 g, 8 g and 14 g.

pavonica [34], the removal of Pb and Cu with activated carbon from *Gracilaria changii* [35] or the removal of Nd by *Ulva lactuca* and *Gracilaria gracilis* [30]. However, past studies do not explore the parameters that shape biosorption in the presence of nutrients in complex mixtures. Herein is presented how different parameters, such as pH, element concentration, nutrients and biosorbent dosage are shaping the biosorption of living *Gracilaria gracilis* for REEs and classical contaminants and its implications for the macroalgae's role in different contamination scenarios.

4.1.1. *Effect of pH and element concentration, and its implications for the applicability of the technology in different contamination scenarios*

The effect of pH should be considered alongside the initial element concentration and nutrient presence to understand, for different contamination scenarios or effluents, the role and limitations of macroalgae during biosorption. Most studies done on biosorption with living macroalgae evaluated the ability of biomass to uptake different classical and emergent contaminants at pH 7.8–8.0 [23,36,37]. However, the fraction of the elements available in solution to interact with the macroalgae might be dependent on a combination of their concentration and pH of the mixture (among other factors such as competing ions) [38]. By studying wider ranges of pH and concentration of elements (Fig. 1), it was possible to evaluate in which contamination scenarios other phenomena, such as precipitation [39,40], will be competing with the macroalgae to bind the metals. This impact is illustrated in Figure S1, in which for the same biosorbent mass (8 g), element concentration (3 μM) and 100 μg of P and 500 μg of N, the contribution of the macroalgae for decreasing element concentration is less relevant at pH 8 than at pH 6. At pH 8, a fraction of the REEs present in the mixture might be forming hydroxide complexes [41] and being, therefore, less available to interact with the macroalgae functional groups (carboxylic, sulphate). At pH 6, these elements are mostly in the form of soluble REE^{3+} ions, allowing the interaction with the negatively charged surface of the biosorbent. Concentrations and speciation of REEs on aquatic systems will depend not only on pH, but also on the redox conditions, salinity, the kind of clay minerals in the surroundings and their complexation with organic and inorganic ligands [42]. The dissolved elements might be highly bound with organic matter, and examples of predominant species are $REE3^{+}$, $REE(SO_4)^{+}$, $REE(CO_3)^{+}$ and REE(DOC) [43], which will be dependent on pH and the stability constants. Moreover, in groundwater of pH = 7–9, the carbonate complexes (Ln $(CO3)^{2-}$ and $LnCO^{3+}$ can be the dominant species [44]. The solubility in waterbodies of these elements, generally, will increase with temperature and decrease with increasing pH, which can also be explained by the precipitation as

Table 4 –
Optimal removal conditions for different scenarios (1, 2 and 3): scenario 1 – Factor A (biosorbent mass) and B (element concentration) are set at any value while Factor C (nutrient concentration) is at maximum level; scenario 2 - Factor A (biosorbent mass) and B (element concentration) are set at any value while Factor C (nutrient concentration) is at minimum level; scenario 3 - Factor A (biosorbent mass) set at minimum value, B (element concentration) and Factor C (nutrient concentration) set at any value.

Element	Optimization scenario	Removal (%)	Factor A (biosorbent mass g L^{-1})	Factor B (element concentration μM)	Factor C (element concentration μM)
As	1	22.0	14	3.42	200
	2	13.9	14	3.89	0
	3	16.6	10.1	2.64	200
Y	1	81.6	14	3.42	200
	2	80.0	14	3.89	0
	3	79.1	10.1	2.64	200
Cd	1	22.8	14	3.42	200
	2	21.7	14	3.89	0
	3	18.2	10.1	2.64	200
La	1	95.0	14	3.42	200
	2	93.1	14	3.89	0
	3	94.5	10.1	2.64	200
Nd	1	93.7	14	3.42	200
	2	95.9	14	3.89	0
	3	92.5	10.1	2.64	200
Eu	1	90.8	14	3.42	200
	2	92.3	14	3.89	0
	3	90.0	10.1	2.64	200
Gd	1	86.8	14	3.42	200
	2	89.2	14	3.89	0
	3	88.1	10.1	2.64	200
Dy	1	87.6	14	3.42	200
	2	87.1	14	3.89	0
	3	87.3	10.1	2.64	200
Pb	1	90.5	14	3.42	200
	2	91.0	14	3.89	0
	3	90.1	10.1	2.64	200
Hg	1	97.6	14	3.42	200
	2	95.9	14	3.89	0
	3	88.3	10.1	2.64	200

carbonates [45,46]. In the pH range of 7.3–8.2, stability constants for common REE complexes will increase with atomic number [46]. Also, the differences in solubility and the competitive interactions with inorganic H^+, Ca^{2+} and Mg^{2+} influence bioavailability [47].

Observing the removal profiles at central point conditions for pH 6 and 8 (Fig. 2), it is noticeable that a rapid initial decrease in contaminant concentrations in solution occurs in the first 6 h of exposure, after which an equilibrium is reached until 24 h, for all the elements presented, except for Cd. This initial behaviour might reflect the metabolism-independent nature of the sorption dominated by interactions such as ion exchange, electrostatic interactions and complexation [11]. At pH 8, other reactions of complexation might be competing with the initial greater availability of binding sites that, usually, are causing a driving force leading to more elevated initial sorption rates [48]. After the initial phase of biosorption, both external and inward interactions simultaneously occur and, finally, internal accumulation of elements prevails [49]. At pH 8, all these dynamics of element-biosorbent and chemical reactions due to the complexity of the mixture might be happening at the same time, being more relevant for higher concentrations (for this mixture >1 μM). These findings point to the importance of technological applications for finding a balance between a suitable pH for living biosorbents and one where the whole fraction of elements is available in solution. *Gracilaria gracilis* showed to be performing with efficiency at a lower pH (6); however, the adequate range might be dependant of the intrinsic biological properties of each species of macroalgae. During the assays, no visible signs of degradation were observed – the tested macroalgae maintained their structural integrity and pigmentation, suggesting that no apparent toxicity occurred. Potential toxic effects of the contaminant mixture were not evaluated since the duration of the biosorption assays was short.

4.1.2. Influence of the presence of nutrients on the removal response by living macroalgae in complex mixtures

Along with pH, it is important to unveil how the bioavailability of the elements in the mixture might be influenced by the presence of nitrate and phosphate, since nutrient enrichment is described to interfere with the metal uptake and accumulation of metals by aquatic organisms [50]. Moreover, from a perspective of water remediation, along with classical and emergent contaminants, nutrients are being discharged into waterbodies. Eutrophication is a consequence of this increasing input of nutrients, such as nitrogen and phosphorus, with adverse impacts on aquatic life [1,6,51]. In the present study, factor C (nutrient concentration) was found to be not significant for the removal of all elements except Cd. For this element, nutrients (C) have a linear negative effect on the removal and a positive linear effect when combined with element concentration (BC), at 48 h. A possible explanation for the presence of N to be related to increased Cd accumulation is the stimulation of the synthesis of amino groups containing ligands [52]. Lee and Wang, 2001) reported that an increase in nitrate concentration resulted in a significant increase in Cd accumulation rate for *Ulva fasciata*. This increase was not always observed for other metals under study, as is the case with the present results. Nutrient enrichment was reported to alter the bioavailability, fate, and distribution of metals in marine organisms (Li et al., 2007b). Nitrate, in particular, can significantly influence metal uptake and accumulation. However, as reported by Li et al. (2007b), the metal affinity order of *Gracilaria gracilis* does not appear to be affected by the presence of nutrients. Investigating the influence of nutrient presence is relevant, as the source of inorganic nitrogen used for macroalgae cultivation can affect the chemical profile of the cultivated species. Consequently, the biosorption performance of macroalgae may be influenced by the nitrate source. For example, B. Shahar et al. [53] showed that the biofiltration performance of *Ulva fasciata* was determined by the form of inorganic nitrogen (N), resulting in inhibition of

nitrate assimilation in the presence of ammonia, for that study.

Quevedo-Ospina et al. [54] reported that nitrogen and phosphorus consumption differed significantly between microalgae cells exposed to Hg and unexposed control cells. The authors found that Hg behaviour within the cell membrane depends on its concentration and is influenced by the structure and amino acid composition of transporter proteins. Cytoplasmic Hg concentrations were associated with the formation of chelating complexes, acting as an active tolerance mechanism.

Table 3 highlights how the variable has a different effect on the response, depending on the element. For the elements Y and La, it has a positive effect, while for Nd, Eu, Gd, Dy, Pb and Hg it has the opposite influence. The metal specificity of the interaction with major nutrients can be attributed to the cationic and anionic nature of the major nutrients. In a previous study, a similar mixture of contaminants, including emergent contaminants such as REEs and trace metals such as Cd, *Ulva lactuca* and *Gracilaria gracilis* had lower removal percentages for this metal when compared to the others in the mixture. If the nutrient supplementation could increase the ability of macroalgae to remove metals that have lower affinities, that would amplify the metal sorption abilities of macroalgae. From the perspective of its efficiency as a biosorbent, as reported by other authors [50], the order of affinity of *Gracilaria gracilis* towards different metals seemed not to be changed by the presence of nutrients. The focus of this study was to evaluate the influence of nutrient presence on the biosorption performance of macroalgae and its environmental implications, considering that complex effluents often reach ecosystems in combination with nutrients. The biological role of phosphorus and nitrate in aquatic organisms, including macroalgae, is widely reported in the literature [55], implying their bioavailability under typical conditions. However, future work could further assess the bioavailability of the nutrients, as depending on their chemical speciation, they may influence macroalgal growth or other metabolic pathways.

4.2. Influence of biosorbent dosage on biosorption performance

Reduced model equations (Table 3) showed which variables were significantly affecting the evaluated response. Overall, the highest removals were obtained in trials with higher biomass dosages, which is corroborated by other literature findings [31]. Better efficiencies are often associated with higher amounts of mass since there are more available active binding sites [56]. For complex mixtures, increasing biomass dosage decreases competition among species present for binding to functional groups of the biosorbent [57].

Fig. 3 shows an overall view of the different response surfaces obtained from the removal performances of different elements. Although it is evident that the biosorbent's dose is significantly affecting the responses obtained, this effect is more accentuated for Pb and Hg than for Y and Nd. Comparing the elements Pb and Hg, it can be observed that the element's concentration is less influential for Pb than for Hg, although for both cases, the influence of the biosorbent mass is more significant. Other authors point out the relevance of the initial biomass, for example, N. Ferreira et al. [31] studied the influence of initial concentration of Hg, salinity, and seaweed stock density in *Ulva lactuca*'s performance and initial seaweed stock density had the most impact on the removal response. In Fig. 4, a comparison between the response surfaces of Hg and Eu is possible. For both, higher biomass doses are associated with higher responses, with the effect more accentuated for Hg (more effectively when the element's concentration is lower). Henriques et al. [24]; E. Fabre et al. [11,23] have been demonstrating the high affinity of *Ulva lactuca* species for Hg removal, even in the presence of other contaminants, and how different operational parameters influence this sorption process. Henriques et al. [24] observed that the effect of ionic strength, evaluated by the variation of salinity, did not affect the ability of *U. lactuca* to remove different metals from water. However, the effect of algal dosage was pointed out as an important parameter to be optimised by the authors, since an increase of biomass allows larger surface areas to be available as binding sites for the contaminants; herein, Hg behaviour showed similarities with literature findings.

For Eu, the removal increases with higher biomass doses and an intermediate value for the element's concentration. Focusing on the response surfaces in which the element concentration is plotted against the nutrient concentration, it can be observed that for Hg, lower concentrations of elements achieved better responses independently of the nutrient concentration used, as for Eu, there is an optimal element concentration and different responses can be obtained with the same amount of nutrients added only changing the concentration of the elements. Finally, focusing now on the surface responses in which the biosorbent mass is plotted against the nutrient's concentration, for Hg and Eu is evident that the effect once more of the biosorbent mass, achieving significantly better responses when the biosorbent doses increase, independently of adding less or more nutrients.

4.3. Optimising removal in complex mixtures in different scenarios

The main objective of the present work was to understand how the studied variables (biosorbent mass, element concentration and nutrient concentration) were shaping biosorption by living macroalgae *Gracilaria gracilis* in a complex mixture of classical and emergent contaminants. From the efficiencies observed, the biosorbent showed more affinity for the classical contaminants Hg (34.0–94.7%) and Pb (39.3–94.7%) and for the REEs (generally above 80%), while As and Cd (less than 50%) were less removed from the solution. From a point of view of water remediation, optimising the removal efficiencies is the most desirable scenario.

Since the biosorbent mass had a significant influence on the removal responses obtained for different elements, optimising or when possible, minimising the amount needed, would have cost benefits if this technology were applied to treat a real effluent. In Table 4, three different optimisation scenarios were explored. In scenario 1, the optimisation was achieved in the presence of the maximum nutrient concentration, while in scenario 2, without their presence; in scenario 3, the target was to minimise the amount of biosorbent used. Overall, for the different optimisation scenarios, the obtained removal responses were very similar; however, for the elements As and Hg, removal responses varied between 16.6 and 22.0 and 88.3 and 97.6%, respectively. For As, with a minimum mass of 10.1 g, the removal response lowered and the optimal element concentration as well. However, for the same optimal mass of sorbent, when nutrients are added, the removal increased from 13.9% to 22.0%. For the other elements, in general, for the same optimum biosorbent mass, adding nutrients does not affect the response. At the same time, with a minimal mass and additional nutrients, the removal responses are also not greatly affected. In a scenario of remediation, the best possible outcome would be with the minimum resources in a time-effective process to be able to achieve high removal responses, even in a complex matrix. From a recovery perspective of the elements of interest, it might be pertinent to selectively remove one element more than the others. However, results seem to point out that the living macroalgae's affinity towards these elements is not greatly changed by the factors studied and, in general, shows great efficiency for the removal of all elements tested, except for As and Cd.

Other biosorbents have been tested for the sorption of REEs, for example, [58] achieved between 80% and 90% of removal for La and Ce with grapefruit peel, while herein, in a mixture of different contaminants in different optimisation scenarios, it was achieved between 93.1% and 92.3%; [59] achieved with walnut shell sorption percentages of $> 85\%$; [60] achieved 92.25% of Y removal from wastewater with *Serratia marcescens*; [61] reported the removal of $> 96\%$ of REEs with *B. subtilis* biomass; [62] reported maximum removal of 41% for Y while and 75–83% for other elements (after 1 h), while 90–92% after 6 h of exposure to titanosilicate materials. Living macroalgae have the advantage of being a cheap and abundant biosorbent, while other

materials might present a high cost in their obtention and present higher technological demands of implementation [63]. Moreover, in the context of water remediation, their lack of selectivity is an advantage, besides the minimal generation of waste at the end of the process, and not requiring chemical activation procedures or other pre-treatments, and being also easily removed from the solution at the end of the sorption.

In Portugal, the discharge limits for Hg, Pb, As, and Cd are defined by national and European legal frameworks, namely Annex XVIII of Decree-Law No. 236/98. The respective emission limit values are 0.05 mg/L for Hg, 1 mg/L for Pb, 0.2 mg/L for Cd, and 1 mg/L for As. Regarding nutrients, the established limits are 15 mg/L for total nitrogen (N) and 10 mg/L for total phosphorus (P). For REEs, no specific discharge limits have yet been established in the current legislation. Considering the removal percentages obtained in the optimised conditions, for the elements Pb and Hg, the final concentrations are below the limits of discharge.

5. Conclusions and future perspectives

The main findings of the study demonstrate that the biosorption performance of living *Gracilaria gracilis* for rare earth elements (REEs) and classical contaminants is strongly influenced by pH, initial element concentration and biomass dosage, while the presence of nutrients did not show to be significant. These results have implications for the relevance of the macroalgae's role in different contamination scenarios and varieties of effluents.

At pH 6, high removal efficiencies were obtained for Hg (96.4%), Pb (94.7%) and the REEs (>80%) after 48 h, except for Cd and As (<50%). Higher efficiencies were achieved with higher biomass dosages, while the presence of nutrients did not significantly affect all elements tested, except for Cd. The non-significant influence of nutrients, from the perspective of applying this technology, might be an advantage for cost savings. At pH 8, complexation with hydroxides is competing with biosorption for concentrations higher than 1 μM. The presence of the macroalgae might not be the only driver of the decrease in the contaminants, competing with the natural or intrinsic processes occurring in the mixture.

Uniquely, this study evaluated the biosorption performance of macroalgae in complex mixtures in the presence of nutrients, at different ranges of pH and initial contaminant concentration, providing insights into the interplay between biosorption and natural processes in complex effluents. Future studies need to evaluate the environmental and economic feasibility of incorporating nutrients as well as the impact of a cultivation step for obtaining this biosorbent, since biomass is a relevant variable in the process.

Funding

This work received financial support from PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UID/50006/2025 – Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos (DOI 10.54499/UID/50006/2025).

CRediT authorship contribution statement

Thainara Viana: Methodology, Investigation, Formal analysis. **Nicole Ferreira:** Methodology, Investigation, Formal analysis, Data curation. **Daniela Tavares:** Methodology, Investigation, Formal analysis. **Jéssica Jacinto:** Writing – original draft, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Bruno Henriques:** Writing – original draft, Supervision, Resources, Project administration, Methodology, Formal analysis, Conceptualization. **Eduarda Pereira:** Supervision, Resources, Project administration, Methodology, Formal analysis, Conceptualization. **Nuno Lapa:** Supervision, Formal analysis, Conceptualization. **João Pinto:** Investigation, Formal analysis.

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.

Acknowledgements

Jéssica Jacinto, Nicole Ferreira, Thainara Viana, and João Pinto thank FCT/MCTES (Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) and ESF (European Social Fund) through NORTE 2020 (Programa Operacional Região Norte) for their PhD grants ref. UI/BD/151290/2021, 2022.13017.BD, 2022.13015.BD and doi:10.54499/2020.05323.BD, respectively.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bej.2026.110216.

Data availability

Data will be made available on request.

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