



**Universidade de Aveiro** Departamento de Química

2020/2021

Pedro Daniel Ribeiro  
da Silva Moleiro

Otimização da biossorção de contaminantes clássicos e emergentes de águas usando *Ulva lactuca*

Biosorption optimization of classic and emerging contaminants from water using *Ulva lactuca*



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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biotecnologia, realizada sob a orientação científica do Doutor Bruno Henriques, Investigador do Departamento de Química da Universidade de Aveiro e da Doutora Eduarda Pereira, Professora Associada do Departamento de Química da Universidade de Aveiro





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## Palavras-chaves

Macroalgas vivas, bioissorção, metais vestigiais, terras-raras, desenho experimental de Box – Behnken, águas

## Resumo

A água é um recurso cada vez mais escasso devido ao stress colocado sobre os recursos hídricos, que resulta do aumento da população humana, de um elevado consumo de água, em grande parte associado à agricultura, e da sua contaminação por atividades antropogénicas. Entre os diversos contaminantes da água, destacam-se, por exemplo, o mercúrio (Hg) (elemento bastante tóxico e perigoso para a saúde humana), o gadolínio (Gd), e o lantânio (La) (dois elementos terras-raras, presentes na maioria dos equipamentos elétricos e eletrónicos). A presença Gd e La nos ecossistemas aquáticos é cada vez mais frequente, sem que ainda sejam bem conhecidos os seus impactos nos organismos. Neste trabalho, a bioissorção e a sua otimização usando a macroalga viva *Ulva lactuca*, é proposta como alternativa promissora para combater o problema da contaminação da água. Nos ensaios realizados (antes de otimização), a remoção dos três contaminantes pela macroalga, em cenários mono- e multi-elementar, apresentou percentagens entre 77 e 88 % para o mercúrio, entre 37 e 53 % para o lantânio, e entre 38 e 65 % para o gadolínio, após 72 h. No ensaio de otimização, a massa da alga (1.5 - 4.5 g/L), a concentração inicial dos elementos (5 – 50 µg/L para o mercúrio e 100 – 1000 µg/L para o lantânio e gadolínio) e a salinidade (15 – 35) foram escolhidas como as condições a estudar usando o Método de Superfície de Resposta com um desenho experimental de Box-Behnken. As condições ótimas de operação que levam à máxima remoção dos elementos (até 100 % de remoção para o mercúrio e para o lantânio, e 94 % para o gadolínio) foram determinadas, e os resultados permitiram concluir ainda que não se justifica estender o processo para além das 72 h. Este trabalho permite preencher algumas lacunas da atual literatura, tal como o estudo da remoção de elementos potencialmente tóxicos de matrizes multi-elementares, em concentrações semelhantes às existentes atualmente no ambiente.

## Keywords

Living macroalgae, biosorption, trace metals, rare-earth elements, experimental design of Box-Behnken, water

## Abstract

Water is a scarcer resource nowadays due to the stress applied to water systems, by the increase of the human population, which leads to a wastage of water mainly due to agriculture and to anthropogenic activities that contribute to the contamination of hydric resources. Examples of relevant water environmental contaminants are mercury (Hg) (a highly dangerous element that is very toxic to human health), gadolinium (Gd), and lanthanum (La) (rare-earth elements, present in most electric equipment and electronics). The presence of Gd and La in aquatic ecosystems is more and more frequent, without being well known their impacts on organisms. In this work, the biosorption and its optimization using the living macroalgae *Ulva lactuca*, was studied to evaluate if it is a promising alternative to deal with the problem of water contamination. In the assays carried out (before optimization), the removal of the mentioned elements in a mono and multi-elementar scenario, achieved removals between 77 and 88 % for mercury, 37 and 53 % for lanthanum, and 38 and 65 % for gadolinium after 72 h. In the optimization test, algae mass (1.5 – 4.5 g/L), initial element concentration (5 – 50 µg/L for mercury and 100 µg/L – 1000 µg/L for lanthanum e gadolinium), and salinity (15 – 35) were the conditions chosen to evaluate the removal using Response Surface Methodology with the experimental design of Box-Behnken. The optimal conditions of the operation that lead to the maximum removal of the elements (until 100 % of removal for mercury and lanthanum, and 94 % for gadolinium) were determined, and the results allow to conclude that the extension of time of the process further than 72 h is not justified. This work fills some gaps of the literature, namely the removal of potential toxic elements from waters in multi-elementar scenarios and in concentrations that are environmentally realistic.



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# 1. Introduction

## 1.1 Water shortage and the need to recycle

Water covers approximately 70 % of the planet Earth, but only 3 % of it is fresh water that can be used for bathing, irrigation, and most importantly, to drink. Nevertheless, two-thirds of the 3 % do not have enough quality for the mentioned uses or is frozen in glaciers, resulting in an even lower percentage. According to the World Wide Fund for Nature (WWF), 1.1 billion and 2.7 billion people worldwide lack access to water and face water shortages for at least one month a year, respectively. Another 2.4 billion are exposed to diseases (like cholera and typhoid fever) derived from inappropriate freshwater quality (1). The United Nations Children's Fund (UNICEF) estimates that one in four children will live in areas of extreme water scarcity by 2040 (2). UNICEF also reported that 2.1 billion people worldwide lack readily available water at home, with 4.4 billion lacking reliable and safely managed sanitation (3). If the consumption rate stays the same, it is predicted that by 2025, two-thirds of the world's population may have to face water shortages with severe consequences to the ecosystems worldwide (1).

So, it is evident the need for an effort to improve the management of potable water, decreasing the misuse of this scarce and precious resource, and to recycle water (wastewater), giving it new destinations (4). The importance of freshwater and water reuse/recycling is evident to UNICEF in The Sustainable Development Goals, with water being a noticeable element to the good health and well-being, clean water and sanitation, sustainable cities and communities, responsible consumption and production, life below water, and life on land being all the stated goals related, directly or indirectly, to water quality (5).

Recycling wastewater implies an improvement in its quality, and allows to extend water supplies, reduce disposal costs and discharges, and save energy. But, recycling water, also has some drawbacks, like the need for treatment plants, water storage facilities, and a delivery system. All these points involve high cost as well. Water subjected to treatment usually contains more salt than most sources of fresh water, which can lead to damage in salt-sensitive plants, crops or groundwater basins (4).

There are various destinations for non-potable water, which include mainly: landscape and crop irrigation, stream enhancement, industrial processes, a barrier to protect groundwater supplies from the intrusion of seawater, wetland habitat maintenance, creation and restoration, and groundwater recharge. In some locations in the state of California, recycled water reached 7 % of the demand for water supply (4).

## **1.2 Water contamination by trace metals and rare earth elements**

The industrialization of modern societies is a factor that negatively affects the environment. In many ways, industrial effluents contaminate rivers and other aquatic bodies by releasing various contaminants in drinkable water bodies (Tiyasha et al., 2020). Because of this, it is important to understand how contaminants interact with the aquatic ecosystem and how and why they can be harmful and hazardous if released unchecked into the surrounding environment.

The main ions dissolved in the aquatic ecosystem are  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^+$ ,  $\text{Ca}^+$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{HCO}_3^-$ . Their concentrations differ between rivers and sea, being much higher in the latter one. Some secondary elements (C, N, P, S, and Si) can occur in water bodies, as well as trace elements, in fluctuating concentrations, depending on various factors, with contamination being the main because their ecological consequences are difficult to predict and to assess with real assurance (Kabata-Pendias and Mukherjee, 2007).

Most of the elements dissolved or associated with suspended particles in water are available to aquatic organisms. They can accumulate in the ocean and rivers, where they are discharged. One of the most harmful groups are trace metals (Kabata-Pendias and Mukherjee, 2007). Trace metals are inorganic elements that can have a nutritional or toxicological significance (Smith and Garg, 2017; Oswell et al., 2019). These elements are commonly associated with toxicity, but some are used as micronutrients in trace concentrations, such as manganese, copper, and zinc (Rodriguez-Freire et al., 2020). These trace metals are mainly discharged by industries. Due to their high toxicity levels, various agencies such as the US Environmental Protection Agency (EPA) and similar ones regulate their discharge and their way of being disposed of (Tchounwou et al., 2012).

Mercury is one of the most toxic and dangerous elements according to various studies, including the US governmental Agency for Toxic Substances and Disease Registry (ATSDR). This agency revises and publishes the “Priority List of Hazardous Substances”, whilst considering the elements’ toxicity, quantity released into the environment, and ease of exposition, being mercury in the 3<sup>rd</sup> position in this list, proving to be a serious health hazard due to the bioaccumulation happening throughout the food chain, potentially reaching us by a source of food or water (Rice et al., 2014; Salazar-Camacho et al., 2021). The toxicity of mercury, at a low-level exposure to organometallic mercury is linked to DNA damage, sterility, retardation in humans, and in animals, leading to kidney damage and reproductive disorder (Kanwar et al., 2020).

Another group of elements that began to raise concerns recently is the rare earth elements (REE). This group is composed of the 15 lanthanides, scandium, and yttrium and has proven to be crucial for day-to-day technological items because of their magnetic properties and defined energy states (Jacinto et al., 2018). Currently, China holds a monopoly on the extraction of these elements (roughly 95 %), which demand is constantly growing. Moreover, the environment is getting contaminated with REE due to mining, and to an improper/inefficient disposal of electrical and electronic equipment waste containing these elements (Carpenter et al., 2015). Two of the most used REEs are lanthanum and gadolinium. The toxicity of these two elements is still a new topic and few studies have focused on them (Moreira et al., 2020; Pinto et al., 2019). In a study performed by Henriques et al. (2019a), gadolinium was claimed to be toxic to mussels (*Mytilus galloprovincialis*), inducing oxidative stress, neurotoxicity, and reducing their metabolic capacity. Regarding lanthanum, for the same species of mussel, it were found biochemical impacts, histopathological alterations, metabolic depression, oxidative stress, and neurotoxicity (Pinto et al., 2019). For the oysters *Crassostrea gigas* it was found that La is among the most toxic compounds for embryos (Moreira et al., 2020).

With the approval of the EU Water Framework Directive 2000/60/EC, some elements/compounds were considered as “priority hazardous substances”, and because of that their discharges should be ceased or eliminated within 20 years. Mercury is one of those elements, which due to its toxicity, has a limit of 0.05 mg/L for the discharge of wastewater, and 1 µg/L



in water for human consumption, according to Decrees-law 236/98 and 306/2007, respectively (same values for Portugal and the European Union). Regarding REE, they are not on the watch list because their toxicity potential is a recent topic of research, although they have already shown potential for toxicity in various species and ecosystems. In the case of gadolinium, it is known that it can accumulate in patients tissue, bone, and, most likely, in the brain (Rogowska et al., 2017). There is also evidence that gadolinium can be assimilated by various aquatic plants and several organisms (*Daphnia magna*, *Lemma minor*, etc.), with negative effects, and this way reach the human food chain (Martino et al., 2016; Migaszewski and Gałuszka, 2015; Rogowska et al., 2017). The same situation applies to lanthanum, proving to be toxic to many living beings such as green algae, and inhibits seed germination as well (Aharchaou et al., 2020; Carpenter et al., 2015). More studies need to be conducted regarding these two elements, so they can be properly recognized and accessed.

A brief description of mercury, lanthanum, and gadolinium applications, sources, and role as a contaminant in the environment is described below.

**Mercury (Hg, CAS n° 7439-97-6)** – The natural sources of mercury are volcanic eruptions, rock weathering, and atmospheric deposition (Kanwar et al., 2020). Mercury is mainly used in electrical industries (switches, batteries, thermostats), mining industries, dentistry (dental amalgams), and has an antifungal agent (Janssen et al., 2021; Tchounwou et al., 2012). According to Fabre et al. (2020b), the concentration of mercury in wastewater can reach 625 mg/L. Porewater in a boreal wetland was studied by Liem-Nguyen et al. (2021), and they found a maximum concentration value of 112.3 ng/L for mercury. In Isonzo River (Pavoni et al., 2020) were measured values of 15.4 and 10.4 ng/L. Janssen et al. (2021), recorded a maximum concentration of 4297 ng/g in particles from in the Mobile River Basin.

**Lanthanum (La, CAS n° 7439-91-0)** – This element is usually found in source rocks and can be in the environment due to natural erosion (Mimba et al., 2020). Lanthanum is used in many industries, such as the glass production industry, in the high-tech industry (present in rechargeable batteries), and in fluorescent lights (Pinto et al., 2019). According to a revision done by Moreira et al. (2020), the concentration of La in the Amazon River was 74.0 ng/L,

increasing in wastewater derived from the mining industry, and 80.4 µg/L for an ore mine in the USA. In a major estuary in France (Lerat-Hardy et al., 2019), concentrations of La ranging between a minimum value of 7.21 ng/L (2003) and a maximum value of 53.0 ng/L (2015) were found.

**Gadolinium (Gd, CAS n° 74440-54-2)** – It is usually found in minerals (ores) consistently associated with other REE (Henriques et al., 2019a). Its natural concentration in river waters was estimated to be, proximately, 1 to 4 ng/L (Rogowska et al., 2017). The applications of gadolinium included alloys, thermoelectric generating devices, electrical car engines, production of nuclear energy, and as a contrast agent in Magnetic Resonance Imaging (MRI) (Rogowska et al., 2017). According to Rogowska et al. (2017), the concentrations in effluents discharged into rivers from Waste Water Treatment Plants (WWTPs) ranged from 200 to 1100 µg/L. In a study by Tepe et al. (2014), it was reported that Gd concentrations in tap water in Berlin (Germany), increased 1.5 to 11.5-fold in just three years. The concentration of Gd continues to increase year by year and it can be seen in a study by Lerat-Hardy et al. (2019), where the values for Gd in a major French estuary went from 12.9 ng/L in 2003 to 20.0 ng/L in 2017.

### **1.3 Removal of water contaminants**

It is imperative to recover the quality of contaminated water. For that, it is crucial to work towards the removal of the toxic compounds at least until their concentration reaches an acceptable level for reuse. The main conventional techniques for trace metals removal are be presented below.

**Precipitation** – This is the process of turning the substance dissolved into its insoluble form or by making the solution supersaturated (Sharma et al., 2019). Chemical precipitation is the most widely used technique in the industry because it is easy to operate with, requires low-cost chemicals and is a simple process to control (Kumar et al., 2021). Chemicals are used to shift the metal ions from a soluble form to an insoluble. Nevertheless, this method has some drawbacks, such as generating large volumes of sludge with disposal problems, and the production of unstable metal hydroxides (Kumar et al., 2021).

Ion exchange – It uses a reversible chemical reaction where an ion in solution is exchanged for an identically charged ion attached to an immobile solid particle (Dahman, 2017). The advantages of this approach for the removal of trace metals from wastewater are the high treatment capacity, high efficiency, and fast kinetics (Fu and Wang, 2011). Ion exchangers may be made by naturally occurring inorganic zeolites or synthetically produced organic resins (Jiménez et al., 2018). However, they seem to be cost-effective only for exchangers such as natural zeolites, and other materials readily available in nature, which can be a downside to synthetic and specific ion exchangers (Entezari and Tahmasbi 2009). This methodology also has issues such as the requirement of high capital investment and secondary sludge disposal problems (Kumar et al., 2021). Another relevant downside is that 70 % of the total costs of the technology are related to costs of operation (Jiménez et al., 2018).

Membrane filtration – This technique has a high efficiency, is easy to operate, and is space-saving. Membrane processes can remove a wide variety of organic, inorganic, and solid particles from surface and sea waters (Teodosiu et al., 2018). Membrane filtration processes include ultrafiltration (UF), reverse osmosis (RO), nanofiltration (NF), and electrodialysis. The choice is based on the size of the particle to remove (Teodosiu et al., 2018). Ultrafiltration has a pore size range of 0.001 – 0.05  $\mu\text{m}$  and an asymmetric membrane. It is used to remove suspended solids, colloids, organics, and bacteria (Singh and Hankins, 2016). Reverse osmosis (RO) uses membranes (also asymmetric) with a pore size of roughly 0.5 nm. This technology is mainly used for the removal of dissolved salts and suspended solids (Singh and Hankins, 2016). Nanofiltration (NF) is the in-between of the two previous cases, it is a pressure-driven membrane with pores having under 2.0 nm in size (Singh and Hankins, 2016). The drawbacks of using filtration as a wastewater treatment are the high initial capital investment needed, and also the secondary sludge disposal problems (Kumar et al., 2021).

Coagulation and flocculation – These two concepts have been defined by Bratby, (1980), where coagulation is a process in which there is the destabilization of the solution, and flocculation is a process by which particles destabilized are bond to form larger structures/particles. These techniques are used in tandem, usual flocculation after coagulation, and, just as precipitation, they are followed by sedimentation or filtration for final removal of trace metals

(Teodosiu et al., 2018). It is mostly used as a pre-treatment in wastewater (Jiménez et al., 2018). The issue with this method is the production of toxic waste of highly concentrated metal and the incomplete removal of contaminants, being most of the times necessary a second treatment after coagulation-flocculation (Jiménez et al., 2018).

Electrochemical techniques – The basic mechanism of these techniques involves the usage of electrochemical properties of the contaminant. When exposed to an external direct current, metal ions are separated from the high concentration solution and are deposited on the cathode (negative electrode) (Zhu et al., 2019). These techniques don't generate secondary waste and don't require the use of additional chemicals (Jiménez et al., 2018). There are several technologies, such as electrodeposition (EC) and electrodialysis (ED) (Jiménez et al., 2018). This technology can only become commercially viable after cost reductions, efficiency improvements, and increase cell life spans, being these the main downsides according to Jiménez et al. (2018).

Sorption – This is a process in which a certain material (sorbate) forms a superficial monomolecular layer on a solid/liquid condensed phase (substrate/sorbent) induced by chemical or physical factors (Crawford and Quinn, 2017). This methodology is recognized as an economic and effective way of water treatment. Sorption is mostly used as a polishing step in the removal of organic and inorganic contaminants in water treatment (Qu et al., 2013). A diverse number of sorbents can be used, such as activated carbon (AC) and carbon nanotubes (CNTs) (Rodríguez-Narvaez et al., 2017; Henriques et al., 2019a). Activated carbon is used due to its extensive surface area and high porosity (Rodríguez-Narvaez et al., 2017), but the high acquisition cost is a disadvantage (Qin et al., 2020). It can still be produced from recycled materials like, for example, tires (Smith et al., 2016). Carbon nanotubes have a large surface area, light mass density, high porosity, hollow structure, and usually a strong interaction with contaminants (Ihsanullah et al., 2016), with removals that can reach 100 % (Rodríguez-Narvaez et al., 2017). A drawback of CNTs is that they have variable performances, even though the same procedure to produce them are followed (Rodríguez-Narvaez et al., 2017). CNTs and other nanomaterials have great potential when comparing to some low specific and low sorption sorbents.

In **Table 1**, the advantages and disadvantages of the different methodologies are summarized.

Table 1 - Advantages and disadvantages of conventional methods for trace metal removal (adapted from Ihsanullah et al. (2016)).

<b>Method</b>	<b>Advantages</b>	<b>Disadvantages</b>
<b>Precipitation</b>	<ol style="list-style-type: none"> <li>1. Simple</li> <li>2. Inexpensive</li> </ol>	<ol style="list-style-type: none"> <li>1. Large volumes of sludge</li> <li>2. Disposal issues</li> <li>3. Ineffective for low concentrations</li> </ol>
<b>Ion exchange</b>	<ol style="list-style-type: none"> <li>1. Metal selective</li> <li>2. High regeneration</li> </ol>	<ol style="list-style-type: none"> <li>1. High cost</li> <li>2. Regeneration can cause secondary pollution</li> </ol>
<b>Membrane filtration</b>	<ol style="list-style-type: none"> <li>1. Less solid waste produced</li> <li>2. Less chemical consumption</li> <li>3. High efficiency</li> </ol>	<ol style="list-style-type: none"> <li>1. High initial and running cost</li> <li>2. Low flow rates</li> <li>3. Removal negatively affected by other ions</li> </ol>
<b>Coagulation and flocculation</b>	<ol style="list-style-type: none"> <li>1. Sludge settling</li> <li>2. Dewatering</li> </ol>	<ol style="list-style-type: none"> <li>1. High cost</li> <li>2. Incomplete removal</li> <li>3. Large consumption of chemicals</li> <li>4. Further toxic wastes and disposal issues</li> </ol>
<b>Electrochemical techniques</b>	<ol style="list-style-type: none"> <li>1. Metal selective</li> <li>2. No consumption of chemicals</li> <li>3. Pure metals can be acquired</li> </ol>	<ol style="list-style-type: none"> <li>1. High capital and running cost</li> <li>3. Initial solution pH</li> </ol>
<b>Sorption</b>	<ol style="list-style-type: none"> <li>1. Most metals can be removed</li> <li>2. High efficiency</li> </ol>	<ol style="list-style-type: none"> <li>1. Cost of activated carbon</li> <li>2. No regeneration</li> </ol>

Although there are several highly efficient technologies to remove contaminants from waters, they all have negative connotations attached, the primary being their high cost (excluding precipitation), incomplete removals, large usage of chemicals and toxic waste generation that have disposal issues. Therefore, it is important to develop alternative technologies that are cheaper, without the disadvantages referred, and that are also environmentally friendly. Sorption seems like a good candidate, if it is discarded the use of activated carbon at a large scale.

## 1.4 Biosorption and bioaccumulation

Biosorption refers to the usage of biomass (usually non-living) for the sorption of contaminants present in a solution. This process is a passive one, occurring metabolically-independent (Fomina and Gadd, 2014). It is also relatively quick, reversible, and, unlike activated carbon, there is the possibility of the regeneration of the biosorbent. According to the literature, any kind of biological material has a certain affinity towards inorganic and organic contaminants, and various studies were aimed towards various biological materials such as seaweed, yeast, bacteria, industrial waste (food wastes and sludge), agricultural waste (fruit waste and rice straw), and natural residues (plant residues and tree barks) (Ayangbenro and Babalola 2017; Henriques et al. 2017a; Kucuker et al., 2017; Kazak et al., 2018; Gupta et al., 2019).

Biosorption depends on the affinity of the organism or biological material (sorber) to the metal (sorbate) (Rodriguez-Freire et al., 2020). Other chemical mechanisms that take place are complexation, chelation, electrostatic and van der Waals forces (Michalak et al., 2013). Most biosorption occurs by binding sites in the cells like alcohols (-OH), aldehydes (R-COH), ketones (R<sup>1</sup>-CO-R<sup>2</sup>), carboxylic acids (R-COOH), and sulfate groups (R-SO<sub>3</sub><sup>-</sup>) (Henriques et al., 2015; Rodriguez-Freire et al., 2020). It seems that biosorption is an economically viable alternative, and it is effective.

Another similar process that goes beyond biosorption, is bioaccumulation, which uses living biomass, having the advantage of accumulating not only in the surface of the biosorbents but also internally (within the cells) (Rodriguez-Freire et al., 2020). Because it takes biosorption a step further it improves the quantity of sorbate collected (Henriques et al., 2015; Henriques et al., 2017a). This process can be segmented into two phases, being the first one like biosorption and the second one is the moving of contaminants to the interior of the cells, mainly through active transport complexes, (metabolically active process) requiring energy consumption (Kumar et al., 2015).

Some downsides in bioaccumulation are the tight control of factors such as pH, temperature, and physicochemical conditions because a live organism is used. It also has to be taken into account that a nutrient source is needed, and the potential stress response caused by the

toxic compound to be removed from solution (Fomina and Gadd, 2014). One of these limitations can be avoided by using photosynthetic organisms – phytoremediation – that are able to use carbon dioxide (CO<sub>2</sub>) as a source of carbon.

A selling point regarding bioaccumulation is the lack of pretreatment of the biomass (there is no need to dry, process, and activate the material (sorbent)), just the simple application of the organism within the contaminant mixture is usually enough. This way of removal works well for trace metals and has been well documented in recent times (Das and Das, 2013; Ayangbenro and Babalola, 2017; Jacinto et al., 2018; Henriques et al., 2019b).

In **Table 2**, there are the main features comparing biosorption with bioaccumulation based on literature.

Table 2 - Comparison between biosorption and bioaccumulation (adapted from Henriques B. (2014)).

Biosorption	Bioaccumulation
Passive process	Metabolically active process
Sorbent is dead biomass	Sorbent is a live organism (biomass)
Metals are attached to the cellular surface	Metals are attached to the cellular surface and in their interior
Main process: Adsorption/ion exchange	Main process: sorption
Nutrients not necessary	Nutrients are necessary
Single-stage mechanism	Double-stage mechanism
Faster than bioaccumulation	Slower than biosorption
Not regulated by the metabolism	Regulated by the metabolism
No toxic effects on biomass	Toxic effects on biomass
Equilibrium with solution is of a higher concentration than in bioaccumulation	Equilibrium with solution is of a lower concentration than in biosorption

### 1.4.1 Biosorbents types

In recent years, the scientific community looked for new and more sustainable ways to remove contaminants from water and, due to this, it created many promising technologies, such as biosorbents. These sorbents can also be separated in various categories.

Natural residues/Biochar – These residues are mainly comprised of matter of the exploitation of forests, processing of its wood, plant biomass, and so on. An example of its use, with

no chemical surface modifications, was reported by Fabre et al., (2019) where unmodified bark of *Eucalyptus globulus* was applied to remove 50 µg/L of Hg in solution. The removal efficiencies varied from 23 to 77 % depending on the pH, salinity, and biosorbent dosage. Another study, also by Fabre et al. (2021), that included bark of the tree *Eucalyptus globulus*, and water hyacinth (dried-up) reported removals of 78.8 % for Hg using *Eucalyptus globulus* bark and of 91.9 % when using water hyacinth, after 72 h. The high availability of these substances is an advantage on its use as a biosorbent.

When the natural waste (mainly plant biomass) or organic matter is pyrolyzed, it is formed a black heterogeneous solid, known as biochar (Hsu et al., 2021; Yin and Zhang, 2020). It can also be produced using seaweed biomass and peels/shells from fruits as well (Inyang et al., 2016; Roberts et al., 2015). In a study performed by Taylor and Husein (2013), a biochar derived from Egyptian mandarin peel was used to sorb 100 mg/L of Hg, being the removal percentages at the end of the process (24 h) between 62.9 and 95.8 %, depending on the pH values on the contaminated solution. In another study by Zhao et al., (2021), it was used straw-derived biochar to adsorb La in three concentrations, 30, 50, and 80 mg/L. After 125 min, the removal percentage reached a plateau of almost 54 %, regardless the concentration.

Agriculture waste – This waste is mainly composed of peels, shells, and husks from fruit/nuts, which can be used in its current state to sorb many pollutants, showing much promise. Regarding this, Fabre et al. (2020b) studied the use of unmodified banana peels for the biosorption of 50 µg/L of Hg, reaching removal values around 92 %. In another case, Fabre et al., (2021) using eggshells, to remove the same concentrations of Hg as the previous work, was able to achieve a removal of 75.7 % at the end of 24 h, and 81.3 % at the end of the process. Using potato peels, the removal was lacking, only being only possible to remove 50.9 % of Hg. There was also the use of grapefruit peel to remove La by Torab-mostaedi et al., (2015). When the concentration of La was 20 mg/L, the removal reached percentages near 95 %. It is also possible to use unmodified nutshells as demonstrated by Dias et al., (2021) to remove Hg, Pb and Cd from multi-element solutions. The removal percentages at the end of the process were 78, 97,



and 98 %, for Hg, Cd, and Pb respectively, for hazelnut. This material has a very high availability due to not being used and having no commercial value (Dias et al., 2021; Fabre et al., 2020b).

Microorganisms – It is possible to use these organisms to remove elements from contaminated aquatic systems. In a paper by Armin et al. (2016), 0.1 g dried biomass of the fungus *Pleurotus eryngii* was used to sorb 1 mg/L of Hg, managing to get a value of recovery of around 60 %, that raised when the biomass increased to 0.35 g (77.4 %). In another study done using *Aspergillus niger* (also dried), Rodríguez et al., (2018) achieved removal values between 30 and 80 %, depending on the initial concentration of Hg used, which varied from 100 to 500 mg/L. In an article by Di Caprio et al., (2016), different strains of *Saccaromyces cerevisiae* biomass were assessed in the removal of La. The best performer was the rim20Δ, managing to get removal percentages between 50 and 88 %. This process can also be done using microalgae, such as Corrêa et al., (2017) did, where the removal percentages of the microalgae mass achieved values around 80 % at the end of the process, both using *Ankistrodesmus* sp. and *Golenkinia* sp. immobilized cells to remove La. There was also an article by Bendakovská et al., (2019), where it was tested the sorption capabilities of a dead microalgae biomass (*Clorella kessleri*) in regards to Gd-contrast agents. The removal varied from around 30 to 90 % at the end of the process, depending on the contrast agent tested. These biosorbents are mostly described as having a low cost and a high affinity to trace metal (Di Caprio et al., 2016; Rodríguez et al., 2018).

Dead seaweed – In recent years, new applications of algae have been studied, with an increasing number of scientific studies conducted (Henriques et al., 2015; Fabre et al., 2020a). In a study by Bendakovská et al., (2019), the ability of dead biomass of *Sargassum glaucescens* (brown algae) and *Gracilaria corticata* (red algae) to remove various quantities of Hg (200 – 1000 µg/L) was tested. The removal percentages were similar between the two algae, around 85 %, with just a slight difference for the lower concentration, where the red algae showed a removal of 70 % and the brown had one around 90 %. In a study performed by Keshtkar et al., (2019), it was used a dried algae, with no pre-treatment and with two pre-treatments (with Ca and with xanthan), to adsorb La. The sorption was greater with the xanthan treatment and lower for the algae who received no treatment, being their respective values of adsorption capacity 97

and 85 mg/g, respectively. The main advantage of using this sorbent is their high ability to bind trace metals due to the functional groups present in their cell walls. It is also a process free from nutrient supply and there are no toxicity constraints. Other upsides of using dead biomass for sorption were the fact that it is eco-friendly, fast, and possibility to regenerate the sorbent, among others (Kadukova, 2016; Mansour and Abou El-soud, 2017).

Taking all these biosorbents stated into account, it is evident that the main downside of using dead biomass is that some of the potential efficiency is lost by not taking advantage of the bioaccumulation process (the accumulation of contaminant inside the cells of the organism) (Henriques et al., 2017a, 2015). This is an aspect that cannot be easily overlooked because it is required a biosorbent which can make full use of its sorption abilities (using both biosorption + bioaccumulation), being at the same time sustainable, cheap, and reliable. The use of microorganisms can also be problematic because of their small size.

**Table 3** presents some examples of efficient biosorbents reported in the literature, as well as the experimental conditions used and target contaminant. It is important to state that in most studies, including the ones in **Table 3**, it is used deionized water or distilled water for the sorption assays, once, due to the lack of ions, it is easier for the sorption to take place.

Table 3 - Examples of used biosorbents, sorption capacities and operational parameters (adapted from Kumar et al. (2014); Gupta et al. (2015); Gisi et al. (2016); Ayangbenro and Babalola (2017); Singh et al. (2020)).

Biosorbent	Metal	Removal capacity (mg/g)	pH	Temperature (°C)
<i>Aeromonas caviae</i>	Cd(II)	155	7.0	20
<i>Oedogonium hatei</i>	Pb(II)	14	5	25
<i>Penicillium chrysogenum</i>	Pb(II)	204	5.5	Not controlled
<i>Thiobacillus ferrooxidans</i>	Zn(II)	172	6.0	40
<i>Bacillus cereus</i> (immobilized)	Hg(II)	104	7	30
<i>B. laterosporus</i>	Cd(II)	159.5	7	25
<i>Micrococcus luteus</i>	Pb(II)	1965	7	27
<i>Spirulina sp.</i>	Cr(IV)	185	7	35
<i>Botrytis cinerea</i>	Pb(II)	107.1	4	25
<i>Cystoseira barbata</i>	Pb(II)	196	4	20
<i>Cystoseira barbata</i>	Hg(II)	32	6.0	Not controlled
<i>Thiobacillus ferrooxidans</i>	Zn(II)	172	6.0	40
Rice husk – treated (PO <sub>4</sub> <sup>3-</sup> )	Cd(II)	2000	8.3	40
Rice husk – treated (H <sub>2</sub> SO <sub>4</sub> )	Hg(II)	384.6	6	45
Wheat straw	Cr(IV)	310.5	2.0	40
Grapefruit peel	U(VI)	140.7	4-6	25
KCl modified orange peel	Cd(II)	125.6	5-5.5	25
Mosambi peel	Cr(VI)	250	2	40
Walnut shell - treated (ZnCl <sub>2</sub> )	Hg(II)	151.5	5	29
<i>Acacia leucocephala</i> bark	Ni(II)	294.1	5	30

### 1.4.2 Living macroalgae as a biosorbent

By using living macroalgae, as opposed to dead biomass, it is possible to go beyond the bonding to the surface and to accumulate internally the contaminants, leading to a higher removal efficiency, which is most relevant to a scaled-up version for the treatment of water from industries (Henriques et al., 2015; Henriques et al., 2017a). The use of macroalgae also proves to be useful when comparing to other live biosorbents such as bacteria and fungi because they don't produce toxins, have large biomass and low nutrient requirements (Okenicova et al., 2016).

Regarding the removal of trace elements such as mercury, there have been encouraging results. Henriques et al. (2015) tested the biosorption capacity of *Ulva lactuca* (green algae), *Gracilaria gracilis* (brown algae) and *Fucus vesiculosus* (red algae) for removing mercury (10, 50, and 100 µg/L), and the removal efficiency varied between 76 and 98 % after 72 h. Studying

the removal of mercury from multi-element solutions, Fabre et al. (2020a) used six different macroalgae, in the presence of REE and potentially toxic elements (PTEs), and it was found that there were negligible effects of the other elements on the removal of Hg for all macroalgae after the 72 h.

A study performed by Jacinto et al. (2018), towards the recovery of lanthanum using the alga *Gracilaria gracilis*, found that it has great removal potential, after 48 h using a concentration of La of 500 µg/L. There was a removal of 50 % in single-element solution and about 70 % in multi-element one, using 2.5 g/L of macroalga (fresh weight). Fabre et al. (2020a) used six different macroalgae (*Ulva lactuca*, *Ulva intestinalis*, *Fucus spiralis*, *Fucus vesiculosus*, *Gracilaria sp.*, and *Osmundea pinnatifida*) to remove REEs from a mixture. High levels of removal of La were achieved in comparison to other elements present in the mixture, between 28 to 68 %, with higher removal for *Ulva lactuca* and the lowest for *Fucus spiralis*. Gadolinium also showed to be highly removed, with efficiencies between 32 – 72 %, with the same algae.

The disadvantage of this technique can be the difficulty in the regeneration of it as a biosorbent, because to recover the contaminant in question inside the cells it is needed to destroy the macroalgae cell walls, damaging the algae severely. Another possible disadvantage is the damage of cell walls by the toxicity of the contaminant, resulting in the loss of cell-binding abilities and the release of contaminant accumulated back to the environment.

There is an evident lack of studies performed on multi-element solutions in literature, as well as studies using concentrations environmentally realistic. Another point worth bringing up is the combination of classic contaminants with emerging ones and evaluate their interactions in solutions whilst studying simple, cheap, and effective ways to remove them from aquatic ecosystems. The selectivity can also be an interesting topic to evaluate, the literature has passed over this topic without much regard for it. The focus will be to uniformize the assays (same pH, same elements, etc.) to allow comparisons to be made between living and non-living macroalgae, to find which one works better for each set of conditions, and to optimize the process.

## 1.5 Influence of experimental parameters on contaminants removal process

Several operational parameters can have an important effect on the contaminant removal from waters, namely:

Temperature - It has an impact on the stability of trace metal ions, the ligands (and the ligand complexes), and on the solubility of trace metal ions, with most chemical reactions being highly sensitive to temperature changes (Kumar et al., 2015). The increase in temperature usually results in an increase in removal due to the expansion of surface area and kinetic energy of the biosorbent that can result from higher temperatures. This is partly due to the increment in heat, increasing the diffusion across the external boundary and in the internal pores because liquid viscosity is lower as the temperature is higher. There is also the fact that the sorption process may be exothermic or endothermic (Ayangbenro and Babalola, 2017). There are studies, conducted by Ayangbenro and Babalola, (2017), that prove this, by increasing the sorption capacity of lead from 0.596 to 0.728 mg/g when the temperature was raised from 25 to 40 °C. It is also fundamental to point out that temperatures too high can cripple the physical structure of the sorbent, and, in the case of living sorbents, tighter control is needed (Fomina and Gadd, 2014). Kumar et al. (2015) did a study of biosorption using microalgae and found lower sorption capacity of cadmium (from 85.3 to 51.2 mg/g) with an increment in temperature. Regarding the removal of neodymium by *Chlorella vulgaris*, Kucuker et al. (2017), reported a lower sorption rate at a higher temperature, claiming that it affected the active binding sites and that most biosorption reactions are exothermic.

pH - The pH of a solution plays a major role in the removal process by governing the speciation of trace metal ions and the surface polarity of the sorbent. The functional groups present on the surface of most sorbents are fully dependent on pH to modulate their affinity to the trace metals in solution (Gupta et al., 2019). Usually, in the case of REE, the studied pH ranges from 1 to 7, since a higher pH results in the formation of insoluble hydroxides due to the hydrolysis of REE (Gupta et al., 2019). Although, there is also some data declaring that sorption performance can increase with increasing the pH of the solution, being the main reasoning the

deprotonation of functional groups, opening more available sites for which the contaminant can attach to. Also, higher pH can be beneficial to the sorption of trace metals because they are positively charged, and by having a solution with high pH in can deprotonate the functional groups of biosorbents, gaining a great affinity towards trace metal sorption. Gupta et al. (2019) showed that the ideal pH range for REE sorption is between 5.0-5.5. For some trace metals, pH can affect its solubility as well as metal chemistry. At low pH, the wall ligands can be associated with H<sup>+</sup> and repulsing the metal ions back. Kumar et al. (2015) presented an improved sorption from 31 to 86 % for copper due to an increase from 1 to 7 in pH. In short, it is understood that pH below 3 should be avoided due to the competition between hydrogen ions and metal ions, and pH above 6.5 can make trace metals to precipitate as hydroxides (Kumar et al., 2015).

Ionic strength – This parameter is an expression of the effect of all ions in solutions according to its electrostatic potential (Gelardi and Flatt, 2016). When it increases, water ionic strength reduces the removal of contaminants through sorption because the trace metals have to compete for the binding spots with other ions (Fomina and Gadd, 2014). At a certain pH, the number of binding sites is set, and by introducing ions to the solution the sites available for the trace metal are reduced (Liu et al., 2019). It also provides a great insight into the complexation mechanism, inner-sphere or outer-sphere complexation mechanisms are known for their sorption of trace metals onto the surface. If the sorption capacity decreases with the increment of ionic strength the process follows the outer-sphere (electrostatic) mechanism. In the inner-sphere (ligand exchange) mechanism, if the ionic strength raises, the sorption can improve or stay unchanged, like the study performed by Liu et al. (2019), where the sorption of copper remained unchanged with ionic strength (inner-sphere). Summing this up, it is crucial to understand how the sorption is taken underway, with cases where the complexation mechanisms switch from outer to inner-sphere by raising pH from 2.5-9 to 9-11 (Gupta et al., 2019).

Initial metal concentration - Usually, the increase in initial contaminant concentration rises the amount sorbed per unit of weight of sorbent, however reducing the removal efficiency (Fomina and Gadd, 2014). Das and Das, (2013), used 25 to 300 mg/L of La in a biosorption study with *Platanus orientalis*, and found that the amount sorbed (mg of La per g of sorbent) was higher when the initial concentration of this metal was too, increasing the sorption capacity

from 9.6 to 57 mg/L. According to this specific element and sorbent, one can say that removal is concentration dependent. If the concentration used is too high, the percentage of removal can decrease because the biosorbent has a limited number of sorption sites, so, there is a limited quantity of contaminant possible to uphold, depending on the biosorbent used. For industrial application, higher removal must occur at lower concentrations for the companies to keep their discharges in check at a low-cost (Das and Das 2013).

Contact time - The contact time is the time that sorbent is in contact with the trace metal that it is supposed to remove. It is a highly variable factor, usually related to the sorbent (and how it operates) and with the trace metal ions. A study by Liu et al. (2019), showed that the contact time leads to an increase in sorption of Cu. The increase was most notably in the beginning of the experiment when most functional groups are readily available to the contaminant. As contact time increases, so do the number of functional groups able to participate in the process (not the number of functional groups itself), until it is reached an equilibrium between the functional groups and the contaminant in the solution. After equilibrium is achieved, there aren't any significant changes in the concentration in solution (Das and Das 2013).

Co-existing ions – Other ions (including other contaminants) are competitors for the same binding sites as the element wished to remove. This means that, in presence of competitors, there is a decrease in the removal of the targeted contaminant. However, loading the sorbent with cations might enhance the sorption of another cation due to pH buffering, in other words, using pH as a targeting mechanism. The presence of anionic species may affect the sorption due to the formation of insoluble metal precipitates, but this depends on the metal speciation, co-existing metals, and the nature of the sorbent (Fomina and Gadd, 2014). This factor is relatively important when considering the industry because, most of the time, industrial wastewater contains more than one type of metal ions (Gupta et al., 2015; Kumar et al., 2015). Like the presence of Ca and Mg in hard waters, and Al, and Fe in industrial effluents are all elements that can compete for the same binding sites and may retard the sorption process for the targeted element. The presence of organic compounds also needs to be accounted for when talking about industrial wastewater. The study on multi-element solutions is most crucial for industrial applications and

to clarify the selectivity of different contaminants, and the quantity sorbed when in solution with other competing ions (inorganic and/or organic) (Gupta et al., 2015).

Another topic that should be addressed is the toxicity of the various elements because if a living organism is being used as a biosorbent, it may not tolerate the toxicity levels of various elements. The effects of these can be synergism, where the combined effect of the mixture is greater than the sum of the individual toxicity of each element; antagonism, where the combined toxic effects are less than the sum of the individual toxicities; or non-interactive, where the combined toxic effects of these elements are like the combined effect. In a particular study, when the algae tested were in contact with 10 different metals (As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Se and Zn) individually, including mercury, no toxicity was observed, but a multi-element system with the same concentration inhibit the sorption by the algae (Kumar et al., 2015).

Sorbent dosage - The sorbent dosage influences the contaminant removal, as increasing its dosage (mass of sorbent per volume of solution), also increases the number of binding sites, incrementing the trace metal sorbed (the interaction between metal ions and binding sites is maximized (same as contact time)). But on the other hand, the quantity of solute sorbed per weight of sorbent decreases, and because of this finding, to study the optimum dosage of the sorbent is crucial for the industry as a cost-effective application (Gupta et al., 2019; Tan et al., 2015). This was demonstrated by Gupta et al. (2019) that observed a decrease in sorption efficiency after a certain sorbent dosage.

## **1.6 Sorbent regeneration**

The regeneration is a must-have feature regarding La and Gd, as REE are extremely valuable, and their desorption and consequent biosorbent regeneration would be a massive advantage. Desorption studies help companies and industries to recover precious metals possibly lost in effluents and regeneration, in literature, helps in understanding the correct method for using biosorbents and decreasing the use/demand of virgin sorbents (Agarwal et al., 2020).

The desorption methodology depends on several factors, such as mechanical strength, sorbent, and the process of sorption. This can be a destructive or non-destructive method, being



in the firstly mentioned, the usage of the dissolution of the sorbent in a strong chemical that will loosen the bond between sorbate and biosorbent, or the combustion of biosorbent. Although it is possible to have high recovery rates, over 95 % in the case of Agarwal et al. (2020), the generation of large volumes of sludge, resulting in an associated disposal cost, can be a downside. The non-destructive technique can be done by physical or chemical treatment. In the physical method, the sorbent is microwaved or heated whilst in the chemical part, it is used metal salts, acids, or alkalis for the recovery to be possible. It must be kept in mind, that for this process not to become destructive it must be used at a low temperature and a low level of acidity. One advantage of this technology, using the chemical way, is that acids and bases are a common waste product in many industries, being the downside of this the loss of some biomass (El-Sayed and Nada, 2017; Agarwal et al., 2020).

Regarding regeneration, various studies included its evaluation for certain sorbents and elements. Such is the case of El-Sayed and Nada, (2017) whereby using a treatment with NaOH it was reached a regeneration extremely high (99 %). Agarwal et al. (2020), showed that with regeneration it was possible to achieve 99 and 95 % of lead and cooper respectively, after pyrolysis of pinecone shell.

## 1.7 Objectives

This work has the aim to evaluate the use of the living macroalgae *Ulva lactuca* to remove mercury, gadolinium, and lanthanum from contaminated water. The specific aspects of this study are:

- To evaluate and compare the capacity of removal of trace metals and rare earth elements such as mercury (Hg), lanthanum (La) and gadolinium (Gd) from single and multi-element solutions at different concentration levels, mimicking environmental conditions.
- To understand the effect of the sorbent dosage, initial metal concentration, salinity, and contact time in the removal of the selected elements, and to compare with each condition's effect.
- To optimize the removal process in regard to the previous stated variables.

## **2. Methods and methods**

### **2.1 Materials and reagents**

All the chemical reagents used in this work had a high purity degree to guarantee the fidelity of the data. All the solutions used in the experiments were obtained by diluting commercial solutions without any purification step. The stock solutions of La and Gd were acquired from Inorganic Ventures<sup>TM</sup> (1000 mg/L in 2 % v/v HNO<sub>3</sub> and 1000 mg/L in 7 % v/v HNO<sub>3</sub>, respectively). The stock solution of Hg was acquired from Merck (Suprapur®, 1000 mg/L in 10 % v/v HNO<sub>3</sub>).

Before being used, all the laboratory glassware was previously washed with distilled water, maintained with HNO<sub>3</sub> 65 % (m/m) for 24 h and re-washed with distilled water. After that, it was filled with HNO<sub>3</sub> 25 % (v/v) during 48 h, re-washed with distilled water and dried. The stock solution of HNO<sub>3</sub> used in washing and for the acidification of samples was obtained from Merck (Suprapur®, 65 % m/m). The seawater used in the assays was collected from the ocean in Barra beach, Aveiro (Portugal, 40°33'N, 8°46'W). Before the experiments took place, the seawater was filtered through 0.45 µm pore size Millipore filter.

### **2.2 Living macroalgae collection and maintenance**

The marine macroalgae used in the assays were collected from Ria de Aveiro, in Aveiro (Portugal, 40°38'N, 8°44'W) and were transported to the laboratory in isothermal plastic bags with some water from the collection site. After that, they were quickly washed with tap water to remove organisms and debris and placed into aquaria filled with seawater (salinity 35), aerated with a pump, under natural light (approximately 12L:12D) at room temperature (20°C).

### **2.3 Contaminant removal assays of Hg, La, and Gd using living macroalgae**

#### **2.3.1 Experimental design**

The sorption capacity of the living macroalgae was assessed by exposing them to mono-element and multi-element solutions of Hg, La, and Gd in seawater, during 72 h. The experiment

was carried out in 0.5 L Schott Duran® flasks containing 0.5 L of contaminated seawater (filtered; salinity 35). Parafilm was applied to cover the top of the flasks with some holes to allow gas exchange with the environment and the macroalgae.

The marine macroalga *Ulva lactuca* was selected for this study, based on its availability, accessibility, and on literature that points it as one of the best performing macroalgae for bio-sorption/bioaccumulation. This seaweed was exposed to three different mono-element solutions of Hg, La, and Gd with concentrations of 50, 1000, and 1000 µg/L, respectively. These concentrations were chosen because it is the maximum approved for discharge, for Hg, and many studies report concentrations in the environment close to the values selected for the La e Gd. The macroalgae was also exposed to two multi-element solutions containing the three elements, where: a) each element is present in the same concentration as the respective mono-element solution; b) each element is present in a concentration ten times lower than that of the respective mono-element solution.

The experiment was done in duplicate, and in the presence of controls (contaminated seawater without algae) to evaluate losses or contamination not related to the presence of bio-sorption/bioaccumulation processes. The pH of all solutions was adjusted with NaOH 1 mol/L to 7.80 – 8.00, which is the usual seawater pH. After contamination and pH adjustment, the solutions rested for 24 h to reach equilibrium. After that, 0.75 g of living macroalgae was added (1.5 g/L, fresh weight) cut in a circular shape. Another flask containing just macroalgae in regular seawater was also run as control (to assess the macroalgae behavior in normal uncontaminated conditions).

The experiment was carried for 72 h, taking water samples (5 and 10 mL) at 0, 1, 6, 24, 48, and 72 h, which were immediately acidified to a pH below 2 with 25 µL of HNO<sub>3</sub> (Suprapur®, 65 % m/m). The samples containing Hg were analyzed right after sampling or frozen at a temperature of -18 °C until analysis. The assay was set up in a shelf near a window with natural light. Before every sample was taken, the flask was agitated to homogenize the solution. After 72 h the macroalgae were collected, weighted and frozen at – 80 °C, for further analysis.

The removal efficiency (%) was calculated using **Equation 1**:

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

Where  $C_0$  is the initial concentration of the contaminant and  $C_t$  is the concentration of the contaminant at time  $t$  (h).

The normalized concentration was also used and calculated using **Equation 2**:

$$Normalized\ concentration = \frac{C_t}{C_0} \quad (2)$$

Where  $C_0$  is the initial concentration of the contaminant and  $C_t$  is the concentration of the pollutant at time  $t$  (h)

## **2.4 Optimization of the removal of Hg, La, and Gd using *Ulva lactuca***

### **2.4.1 Experimental design**

After assessing the potential of *U. lactuca* to remove La, Gd, and Hg in both mono and multi-element scenarios, the optimization of the process was investigated for the multi-element system, as it is closer to real cases. The experiment lasted for 120 h, using the same volume of contaminated solution on the same brand of 0.5 L flasks (Schott Duran®). A Box-Behnken design (**Table 4**) was followed, which considers three factors in three equidistant levels. This means that all the extreme conditions tested must be at an equal distance from the medium value. In this work the factors or parameters investigated were salinity, mass of algae, and initial concentration of the element. The values for the mass of *Ulva lactuca* were 1.5, 3.0, and 4.5 g/L, for the water salinity were 15, 25 and 35, and for elements initial concentration were 5, 27.5, and 50 µg/L for Hg, and 100, 550, and 1000 µg/L for La and Gd.

Besides those fifteen conditions (**Table 4**), five more were done, one with just macroalgae and seawater (salinity 35) to serve as a blank, and the other four to serve as control (contaminated solution with no algae) to test the loss of contaminant without the presence of the biosorbent (through volatilization and/or sorbed to the flask walls) and possible contaminations. Thereby, two of these had the lowest concentration with salinity of 15 and 35 and the other two with the highest concentration tested with salinity of 15 and 35 as well. The pH of seawater was measured (8.11) and each condition had their pH adjusted with NaOH 1 mol/L to 8.00 – 8.15.

Table 4 - Experimental conditions tested using the Box - Behnken design. The fixed conditions were the pH (7.8 - 8.0), contact time (120 h), and volume (0.5L).

Condition	Macroalgae mass (g/L)	Hg ( $\mu\text{g/L}$ )	La ( $\mu\text{g/L}$ )	Gd ( $\mu\text{g/L}$ )	Salinity
1	3	5	100	100	35
2	3	27.5	550	550	25
3	1.5	27.5	550	550	15
4	4.5	50	1000	1000	25
5	3	5	100	100	15
6	3	27.5	550	550	25
7	4.5	27.5	550	550	35
8	3	27.5	550	550	25
9	4.5	5	100	100	25
10	4.5	27.5	550	550	15
11	1.5	27.5	1000	1000	35
12	3	50	1000	1000	15
13	3	50	1000	1000	35
14	1.5	5	100	100	25
15	1.5	50	1000	1000	25

As beforehand, the contaminated solutions were prepared and left for 24 h to achieve equilibrium, and then, the algae weighted for each specific experiment was added, previously cut in a circular shape. Water samples (5 – 10 mL) were taken at 0, 24, 48, 72, and 120 h, and immediately acidified to a pH below 2 with 25  $\mu\text{L}$  of  $\text{HNO}_3$  (Suprapur®, 65 % m/m). After this, the samples that contained Hg were covered with parafilm and then put in the freezer (- 18 °C) until analysis or were immediately analyzed after collection. The other samples were also stored after and covering with parafilm. The experiment was assembled near a window with natural light. Before every sample was taken, the flask was agitated to homogenize the solution.

Data (% of removal) were analyzed using the Response Surface Methodology (RSM), a tool (using Design Expert version 13, trial version) that serves to outline the connection between several independent factors and one/more responses. The RSM is based on several models such as quadratic, linear, cubic, among others) in order to fit a certain number of results from the Design of Experiment (DoE), whilst verifying the model proposed using statistical means. The objective of using the DoE is to optimize the process (the efficiency) whilst keeping to minimum the time of the assay and the costs attached to it (Fabre et al., 2019).

## **2.5 Water analysis**

### **2.5.1 Quantification of mercury**

The quantification of Hg in water was performed using a cold vapor atomic fluorescence spectroscopy (CV-AFS), PSA 10.025 Millennium Merlin Hg analyzer and SnCl<sub>2</sub> (2 % m/v in HCl 10 % v/v) as a reducing agent. Five standard solutions (0.0, 0.1, 0.2, 0.3, and 0.5 µg/L) were prepared daily to calibrate the equipment (calibration curve accepted always had a R<sup>2</sup> higher than 0.9950). At least, three replicate measurements were performed out of every sample. The quantification limits were determined as the concentration of the lower standard, that is 0.1 µg/L.

### **2.5.2 Quantification of lanthanum and gadolinium**

The quantification of La and Gd in solution was done at the Laboratório Central de Análises da Universidade de Aveiro (LCA-UA). This institution is accredited for the analysis of waters and soils using an inductively coupled plasma optical emission spectrometry (ICP-OES). All the liquid samples were analyzed by ICP-OES.

The ICP-OES equipment used was a Horiba Jobin Yvon, Activa M Quadrupole Thermo Scientific X Series, being the quantification limits for both Gd and La 10 µg/L.

## **2.6 Quantification of chlorophyll**

For the quantification of chlorophyll, it was used the Chlorophyll Meter SPAD – 502Plus, from Konica Minolta. The device is non-destructive and lightweight, used to determine

the relative amount of chlorophyll. It measures the absorbance in two wavelengths, blue (400 – 500 nm) and red (600 – 700 nm), and, with these, it calculates a numerical SPAD value, being this proportional to the amount of chlorophyll present in the sample tested (leaf, algae, ... ) (Konica Minolta, 2017). It has been proven that this equipment is of an extremely high fidelity in its readings (Shibaeva et al., 2020). In the present study, three to four macroalgae disks of each condition were analyzed (5 readings each).

## 2.7 Quality control in the data treatment

### 2.7.1 Control cards

One method to guarantee the quality and fidelity of the data is through control charts. The objective of these charts is to verify if the data follows the quality requirements necessary (warning and rejection lines in **Figure 1**). In this study, the control charts were elaborated to evaluate the concentration of different controls (contaminated solution without the presence of biosorbent) for each element. The values were expressed as normalized concentration ( $C_t/C_0$ ) and the warning and rejection lines were created considering deviations of 10 and 20 % from the initial value ( $C_0$ ), respectively.

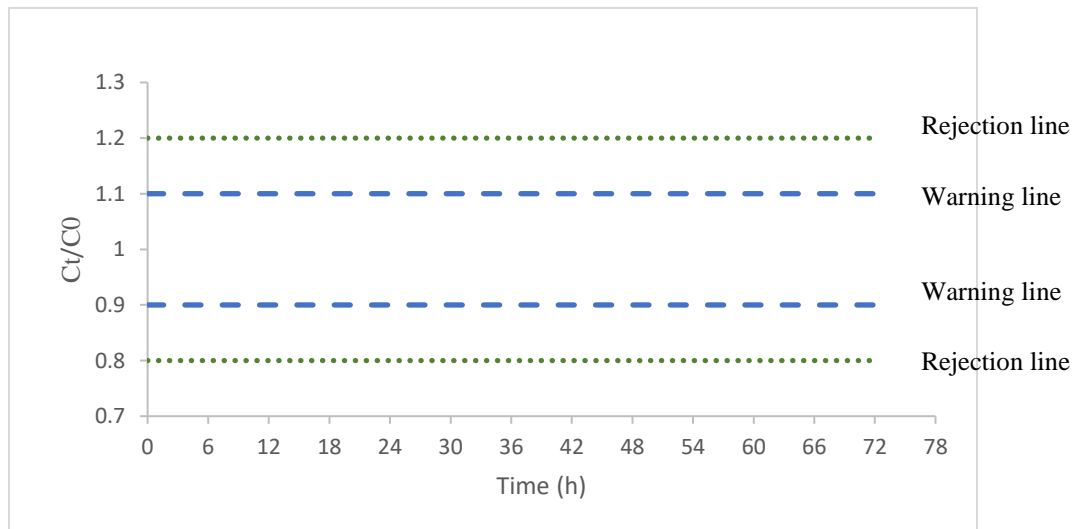


Figure 1 - The structure of control cards

## 3. Results and discussion

### 3.1 Control charts of the assays with Hg, La, and Gd

To ensure the quality of the results of this set of experiments, i.e., to ensure that no external factors besides the biosorbent used were responsible for the removal and biosorption of the contaminants, data from controls (contaminated water with no macroalgae) were analyzed. Constant values throughout the whole experience for the control are expected (meaning absence of contamination or loss). If this assumption is not verified, the  $C_t/C_0$  values fall out of the lines stipulated. If the line is the rejection line, the values are not considered for the assay. The control charts are showed in **Figure 2**. All the values regarding Hg fall within the rejection lines except the values at 48 h in A (1.23) and B (1.22), which were very close to the limit stipulated of 1.20, both having an initial concentration of Hg of 50  $\mu\text{g/L}$  and the value at 48 h in C (1.39), whose deviation was greater. This latter example may be justified by the very low concentration used (5  $\mu\text{g/L}$ ), where it is more difficult to control loss or contamination phenomena.

For La, all the values fall withing the warning lines apart of a value at 24 h that is between the warning and rejection lines (E – 0.88). The control for mono-element solution of La (D) shows that this element tends to precipitate at relatively high pH (above 6) when it is alone in solution. Because of this, the removal of La by *U. lactuca* in mono-element solution cannot be properly evaluated, and it was not shown. Lanthanum can form complexes with other elements present in solution (Jaireth et al., 2014; Pinto et al., 2019), which are soluble at pH above 6. This was observed in the present study (control cards E and F in **Figure 2**), enabling to evaluate the removal of La using *Ulva lactuca* in multi-element solution.

Regarding the control charts for Gd, all the values fall within the warning lines, with just one of the values surpassing it, 1.12 (**Figure 2** H). Taking all this into consideration, the quality of results is assured, and Gd data can be used to reach further conclusions and hypothesis.



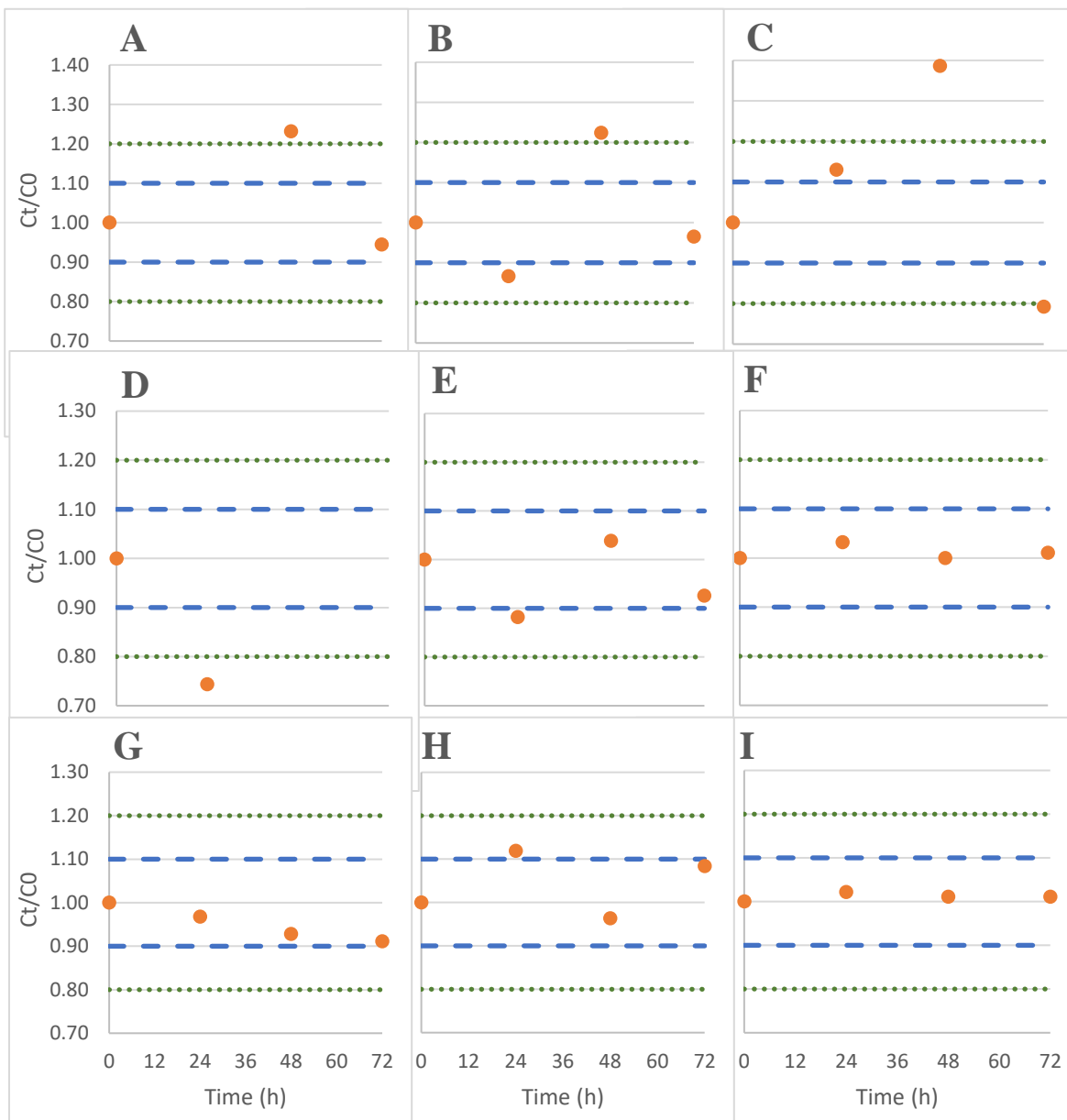


Figure 2 - Control charts with normalized concentration ( $C_t/C_0$ ) values for the controls at 0, 24, 48, and 72 h. The conditions represented are as follows: A) mono-element solution of Hg (50  $\mu\text{g/L}$ ); B) multi-element solution of Hg (50  $\mu\text{g/L}$ ); C) diluted multi-element solution of Hg (5  $\mu\text{g/L}$ ); D) mono-element solution of La (1000  $\mu\text{g/L}$ ); E) multi-element solution of La (1000  $\mu\text{g/L}$ ); F) diluted multi-element solution of La (100  $\mu\text{g/L}$ ); G) mono-element solution of Gd (1000  $\mu\text{g/L}$ ); H) multi-element solution of Gd (1000  $\mu\text{g/L}$ ); I) diluted multi-element solution of Gd (100  $\mu\text{g/L}$ ). The blue and green traced lanes are the warning and rejection lines with a deviation of 10 and 20 % from the initial value ( $C_0$ ), respectively.

### 3.2 Removal of Hg, La, and Gd using *Ulva lactuca*

The macroalga *Ulva lactuca* was used to remove Hg, La, and Gd from water, under 5 different conditions, including mono- and multi-element solutions of these three elements. The

concentration of Hg chosen corresponds to the maximum amount approved by legislation regarding discharged effluents. Gadolinium and La do not have yet regulated values for wastewater discharge, but concentrations between 1  $\mu\text{mol/L}$  (139  $\mu\text{g/L}$  for La and 157  $\mu\text{g/L}$  for Gd) and 500  $\mu\text{g/L}$  have been used in some previous published studies. In the present work, 1000  $\mu\text{g/L}$  for La e Gd was chosen because it is known that the lack of regulations makes discharges of these elements uncontrollable, and it is expected an increase in the concentrations of these REE in the environment in the near future (Fabre et al., 2020b; Jacinto et al., 2018). A contamination scenario with the elements in a concentration 10 times lower was also studied to mimic what is possible to observe nowadays in the aquatic environment (i.e., the dilution of the wastewater at the discharge point in the aquatic ecosystem).

**Figure 3** presents the evolution of the normalized concentration of Hg and Gd during the 72 h the experiment took place, for the mono-element solutions. The control tests are represented by the dotted lines (contaminated water without the biosorbent).

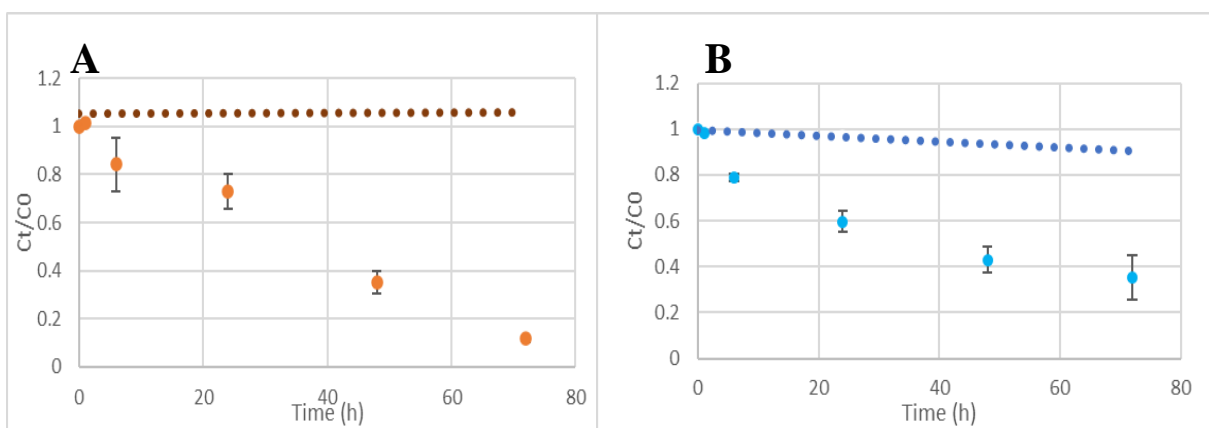


Figure 3 - Normalized concentrations (Ct/C0) for the mono-element solutions of A) Hg, B) Gd. The traced line represented the controls. The error bars are standard deviation between replicates.

According to **Figure 3**, it is evident that the removal process is fast, being able to reach high removals after 72 h. It is also possible to see that the equilibrium between the contaminants in solution and the contaminants on the surface of the macroalgae was not achieved within the exposure time. In all elements, it is also noticeable a decrease in concentration after 6 h of exposure, being this decrease higher for Gd (0.79) than for Hg (0.84), having the macroalgae being able to sorb 210 and 8  $\mu\text{g/L}$ , respectively. After 72 h, regarding Gd, a concentration of only 350

$\mu\text{g/L}$  remained in the solution. Regarding Hg a concentration  $6 \mu\text{g/L}$  remained in the solution after 72 h. In the beginning, *Ulva lactuca* showed a higher affinity towards Gd, but at the end of the assay the affinity looked greater to Hg, both these cases regarding their respective algae in the mono-element solution. These aspects can be explained due to the presence of functional groups on the algae surface, mainly sulfur groups, which can lead to a high affinity towards Hg than to Gd (REE), as stated by Fabre et al. (2020a). This result is also in concordance with several articles regarding the same topic, where Hg is preferred by the macroalgae than other elements, such as REE or other Critical Rare Elements (CRE) (Henriques et al., 2019, 2017a, 2017b).

**Figure 4** presents the removals obtained in all the conditions tested, after 72 h. It is notable that the removal of Hg reached high values, 88 % in mono-element solution, 82 % in the mixture with the other two REE, and 77 % in the diluted mixture. It is also possible to conclude that the removal of the REE is lower (38 % for Gd and 37 % for La) in the less concentrated mixture. The highest removal efficiencies for Gd were observed in mono-element solution (65 %). The highest removal for La, discarding the mono-element solution which didn't prove to be viable, was in the multi-element solution (53 %). With this, it is important to note that an increment of the initial contaminant concentration affects positively the final removal percentages. This behavior is expected due to the sorption being influenced through the concentration gradient between the contaminated solution and the macroalgae, so, higher concentrations, tend to increase the removal of the contaminant. It is possible to conclude that the saturation level wasn't achieved because when the concentration increased, so did the removal of all three elements. This process keeps occurring until a chemical balance is established between the surface of the sorbent and the solution. This hypothesis is in concordance with Henriques et al. (2015), where the biosorption and bioaccumulation of Hg (three different concentrations of Hg, 10, 50, and  $100 \mu\text{g/L}$ ) was tested using three marine algae (*Ulva lactuca*, *Gracilaria gracilis*, and *Fucus vesiculosus*). It was verified, that the percentage of removal of this element for

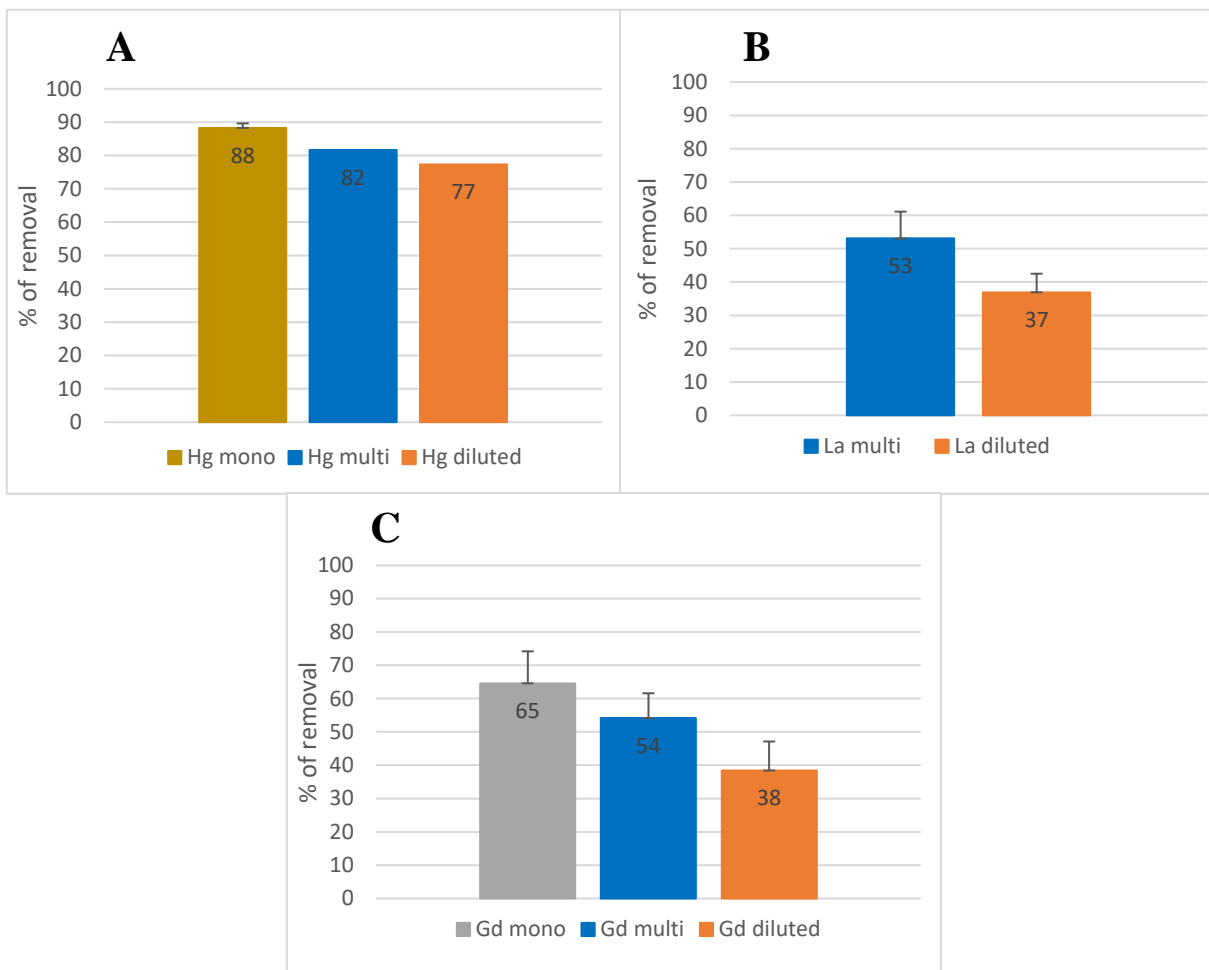


Figure 4 - Removal (%) using *Ulva lactuca* in all conditions tested after 72 h for A) Hg, B) La, and C) Gd. The error bars are standard deviation between replicates.

all macroalgae tested was greater the higher the amount of Hg present initially in the contaminated solution, justifying this by hypothesizing that the enhanced uptake could be derived from the greater driving force that higher concentrations provide, overcoming the mass transfer resistances between Hg in the solution and the macroalgae. This hypothesis is also mentioned on another study regarding the bioaccumulation of Hg, Cd, and Pb using *Fucus vesiculosus* performed by Henriques et al. (2017a). If the macroalgae can withstand the toxicity of these elements, higher concentrations can be used to promote greater removals. If the growth rate is not compromised, the removal increases due to the number of sorption sites on the surface also increasing, being advantageous when compared to dead algae (Henriques et al., 2015).

Regarding multi-element solutions, the affinity for Hg is still unchanged, but the affinity towards Gd is different, being similar to La in this type of solution, having removal percentages for La of 53 % and 37 %, and 54 % and 38 % for Gd in the multi-element and diluted multi-element solutions, respectively. This fact, makes these two elements indistinguishable to the macroalgae, having a similar affinity to be sorbed in the presence of other contaminants as well. In a study already mentioned conducted by Fabre et al. (2020a), six marine macroalgae, *Ulva lactuca*, *Ulva intestinalis*, *Gracilaria* sp., *Fucus spiralis*, *Fucus vesiculosus*, and *Osmundea pinnatifida* were used to remove Hg and other REEs (including La and Gd) from a multi-element solution. The concentrations tested were 1  $\mu\text{mol/L}$  (200  $\mu\text{g/L}$  for Hg 139  $\mu\text{g/L}$  for La, and 157  $\mu\text{g/L}$  for Gd) for all the contaminants. Turning the focus on *Ulva lactuca* and regarding Hg, La, and Gd, the removal percentages reported were different depending on the contaminant, being the greatest for Hg (94.0 %), then for Gd (71.8 %), and finally La (68.0 %). It was concluded that the REEs have a low affinity towards the S – H groups on the algae surface, unlike Hg (Fabre et al., 2020a). It is also known that the decreasing atomic ray is related to the decrease in sorption regarding REE (Fabre et al., 2020a). It is also determined that, in the scenario portrayed by the study cited, the presence of other contaminants does not significantly affect the removal percentages compared to mono-element solutions (85 – 99 %), to multi-element solutions with PTEs and REEs (85 – 93 %). This seems to be the case, due to the removal of Hg changing from 88 % when isolated to 82 % when in conjunction with other elements in low/realistic concentrations of the present study. Gadolinium follows the same trend, where the difference between mono and multi-element solutions was 11 % (65 – 54 %).

### 3.3 Optimized conditions for the contaminant's removal

#### 3.3.1 Removal of Hg, La, and Gd in mixture using *Ulva lactuca*

After assessing the potential of *U. lactuca* to remove Hg, La, and Gd in different contamination scenarios, the next step was to optimize the experimental conditions to maximize the removal, using a Box-Behnken design. Various parameters in the experiments were changed to find the best combination, regarding salinity, initial element concentration, and macroalgae (bi-sorbent) mass. The removal percentages observed in the three replicates of the central point (where all the parameters had the central value), experiment 2, 6, and 8 (**Table 4**), are represented in **Figure 5**.

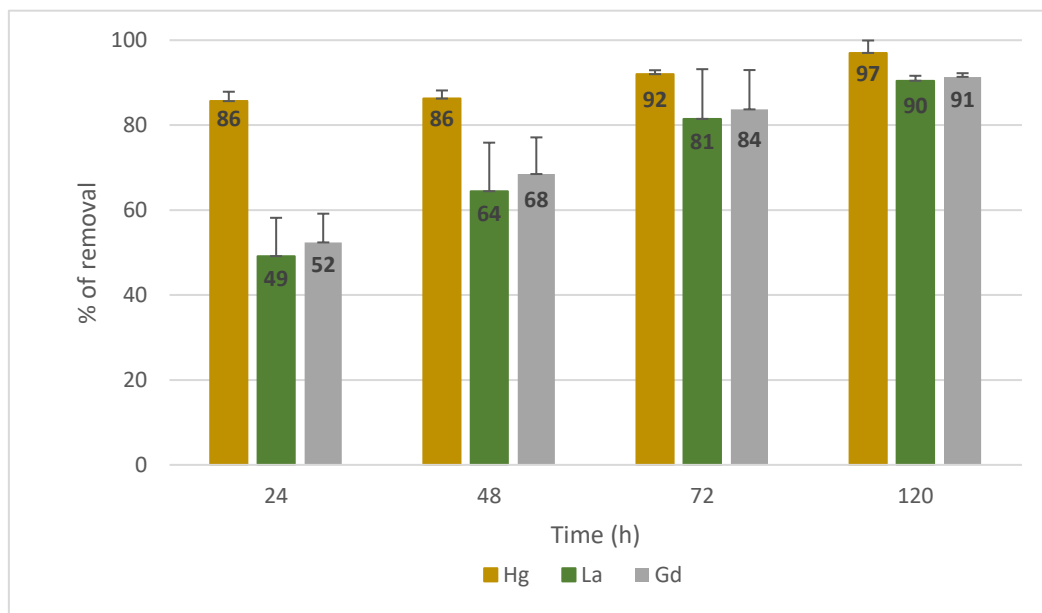


Figure 5 - Removal (%) mean value using *Ulva lactuca* for the central point after 24, 48, 72, and 120 h for Hg, La, and Gd. The central point conditions are: 3.0 g/L of macroalgae biomass, salinity of 25, and 27.5 µg/L for Hg, 550 µg/L for La and Gd. The error bars were calculated using each experiment as a replicate.

It is assumed that the experimental design followed is not viable if the precision (error bars) of the central point results exceeds 10 %. This benchmark was not crossed, assuring the viability of the Box-Behnken design. Adding to that, the control results (not shown) remained constant and within the rejection lines throughout the experiment, between 0.81 and 1.10 ( $C_t/C_0$ ). The removal percentages for the 15 conditions evaluated, after 120 h, can be found in **Table 5**. The results show a notorious removal of Hg in all the experiments conducted, being between 88 and 99 %.

Table 5 - Removal percentage after 120 h for Hg, La and Gd of the experiments using the Box-Behnken design.

Experiment	Removal of Hg (%)	Removal of La (%)	Removal of Gd (%)
1	89	90	90
2	94	89	91
3	89	71	73
4	98	74	75
5	97	80	80
6	98	90	91
7	96	76	77
8	99	92	92
9	88	92	89
10	97	72	72
11	90	68	71
12	98	67	75
13	97	86	88
14	94	68	72
15	93	61	62

Regarding La and Gd, although lower than the previous examples, the assays also show high removal percentages. The same trend of the previous experiment can be seen here, where the removal percentages of Hg are much higher than those of the other two elements, once again showing the high affinity of *Ulva lactuca*'s functional groups towards Hg ions. The two REE have a bigger range in the removal percentage than Hg, between 61 and 92 % for La, and between 62 and 92 % for Gd, compared to 88 – 99 % for Hg. Another noticeable point is that La and Gd have very similar removals when the same parameters were tested. This is in concordance to what was noted in the previous trial, where the properties of these two REE are more related to each other and are more similar when in presence of each other and/or in a multi-element environment. Taking a close look at the following results (**Figure 6, 7 and 8**), is evident that all of them are characterized by a very high removal speed in an early phase, from 0 to 24 h. The speed decreases as the time of the experiment also increases, with some reaching a plateau at the 120 h mark. The more evident one is with Hg (**Figure 6**), which in most cases the removals during the first 24 h reached values of normalized concentration between 0.46 and 0.07. In the last 48 h, most of the removal increased just by 0.01 and 0.09, being the only outlier the Experiment 10 (4.5 g/L of algae mass, salinity of 15, and 27.5 µg/L of Hg, 550 µg/L of La

and Gd), that further decreased the concentration in 0.26. This happens due to the first, most dominant process, biosorption, which is characterized as fast, superficial, passive, and governed by adsorption and/or ion exchange. After this, the most dominant process becomes bioaccumulation, where there is the absorption of contaminant by the macroalgae, which is more slow, and metabolic-dependent (Kumar et al., 2015).

Another thing worth noting is that these results closely resemble those of the first trial undertaken. This trend is concurrent with other articles and studies, such as the of Henriques et al. (2015), where the removal of 100, 50, and 10  $\mu\text{g/L}$  of Hg using 1.6 g/L of *Ulva lactuca* was tested. In this study, it is stated that the trend in the removal is due to the early stage being a metabolic-independent process, with the macroalgae surface free from Hg ions, prompting a faster process in the beginning.

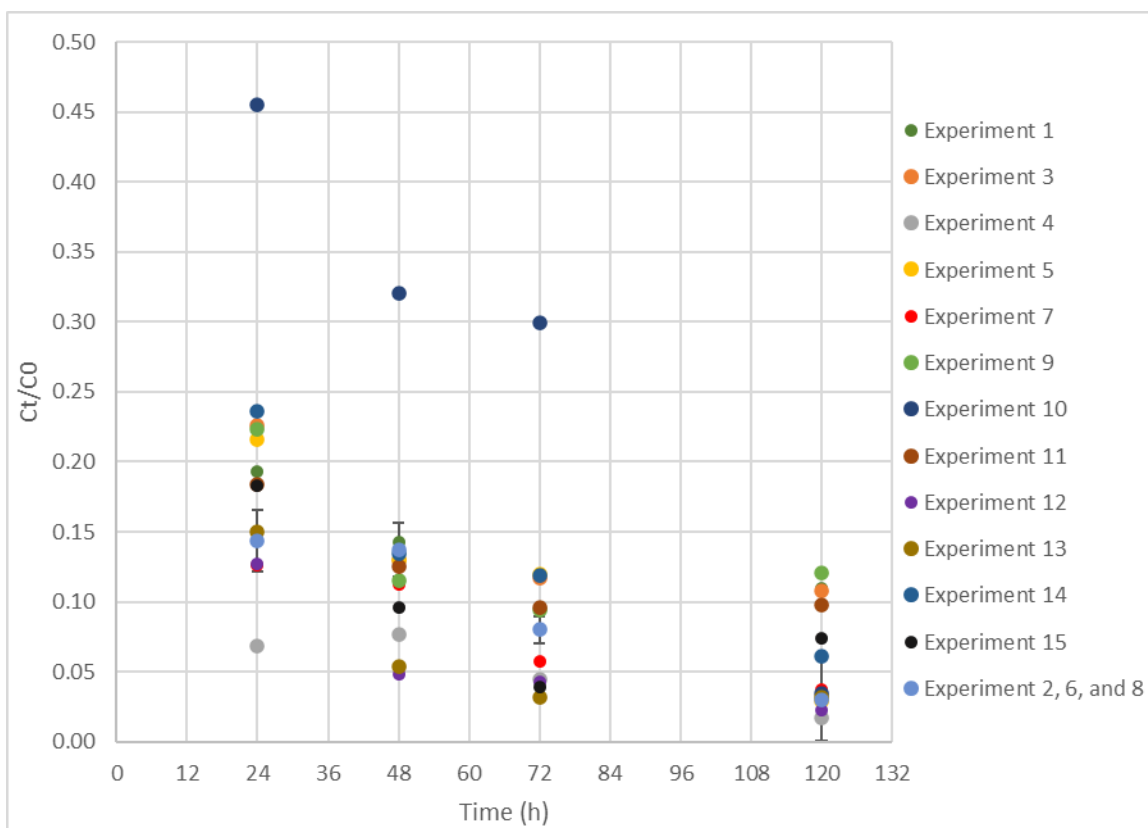


Figure 6 - Normalized concentrations (Ct/C0) for Hg of all 15 experiments for 120 h. The error bars are standard deviation between replicates.



Another element to consider, observing the **Figure 6**, is that pretty much all experiments showed very low removal of mercury in the last 48 h, more precisely between 72 h – 120 h. This shows that the macroalgae surface was still not fully saturated with Hg ions but might very close to being. This could happen due to the gradient between matrices (solution and algae surface) being more similar in later stages, according to the results shown. Most of the concentrations vary from 0.01 to 0.09, although in some cases there has been an increment in Hg concentration in solution when compared to 72 h. In most cases, there is still the chance to increase the removal of this element possibly further in contaminated waters.

An important factor in this experiment is that in some cases the removal led a final concentration of Hg in solution lower than 1 µg/L, a concentration equal to the maximum allowed for the water to be classified as drinkable for people. Not only this shows the high capacity of the macroalgae to remove Hg from contaminated and realistic aquatic environments, but also shows another process that can promote the reuse and recycling contaminated water turning it into “new” potable water.

Regarding La and Gd, in **Figure 7** and **Figure 8** respectively, the same can be said, but at a lower degree, the removal occurs in its majority during the first 24 h of contact, leading to normalized concentrations from 0.25 to 0.85 for La, and for Gd between 0.22 and 0.81, being this an improvement when comparing to the first assay. But there are also large quantities of La removed in the last 48 h of the experiment, something not so notably shown in the removal of Hg, achieving removals increments between 0.02 and 0.65. This last statement also applies for Gd, where it was attained a removal during the last phase from 0.01 to 0.73. Regarding these two elements, both had certain conditions which show a negative/neutral removal between 72 h and 120 h, being the cases similar for the two REE. A possible explanation can be due to human mistake in sampling/quantification or, it is possible that the macroalgae could not handle the toxicity of the elements and began to decay, releasing these elements back into the solution, due to the difference in gradient. Although Hg affinity and removal percentages are relatively high in comparison with the other tested REE, its removal (in terms of concentration) was lower. So, in the case of this study, using Experiment 8 (3.0 g/L of algae mass, salinity of 25, and 27.5 µg/L of Hg, 550 µg/L of La and Gd) as example, it was able to remove 99.0 % of mercury, meaning

27  $\mu\text{g/L}$ , regarding La and Gd the removals were 92 % (506  $\mu\text{g/L}$ ) and 92 % (506  $\mu\text{g/L}$ ). Although the removal percentages were higher in the first case, the quantity removed was much lower in comparison to La and Gd. This is mainly due to the concentrations tested but can also be prove that this process can provide the crucial recycling of these high value elements, to be reintroduced into the market by remediating contaminated waters.

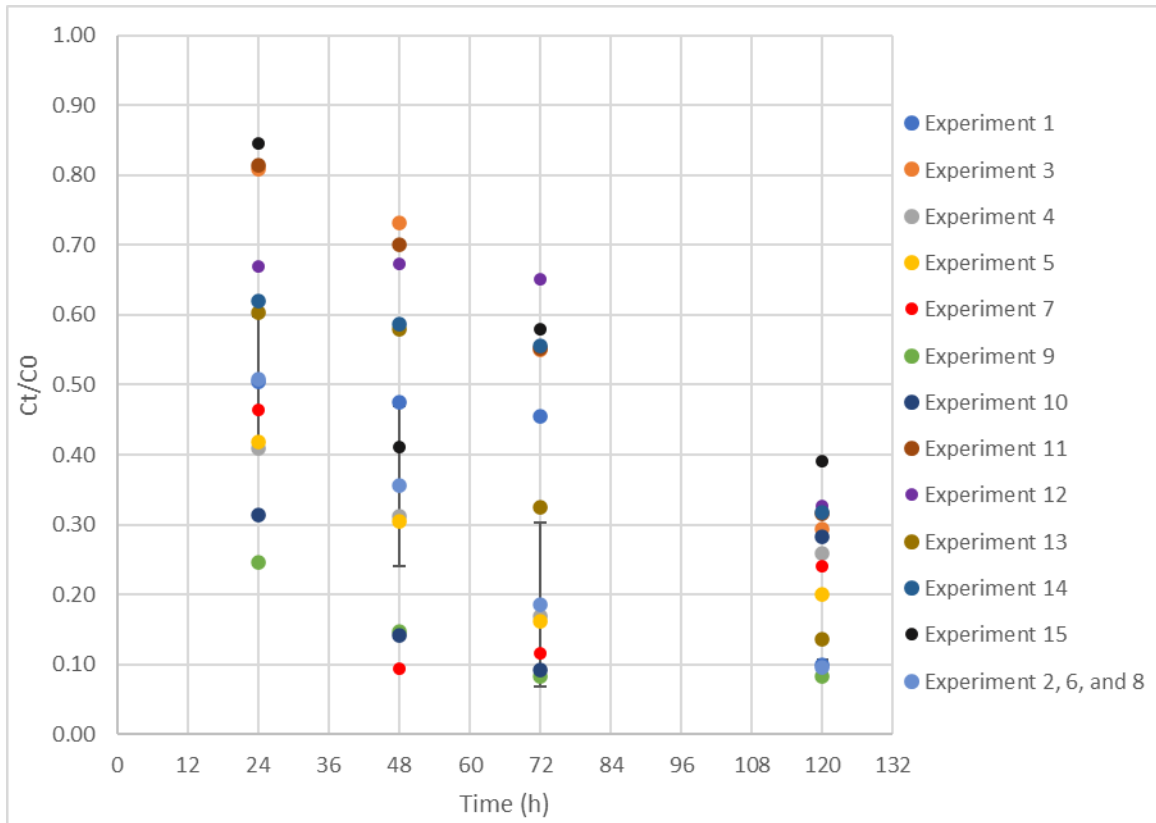


Figure 7 - Normalized concentrations ( $C_t/C_0$ ) for La of all 15 experiments for 120 h. The error bars are standard deviation between replicates.

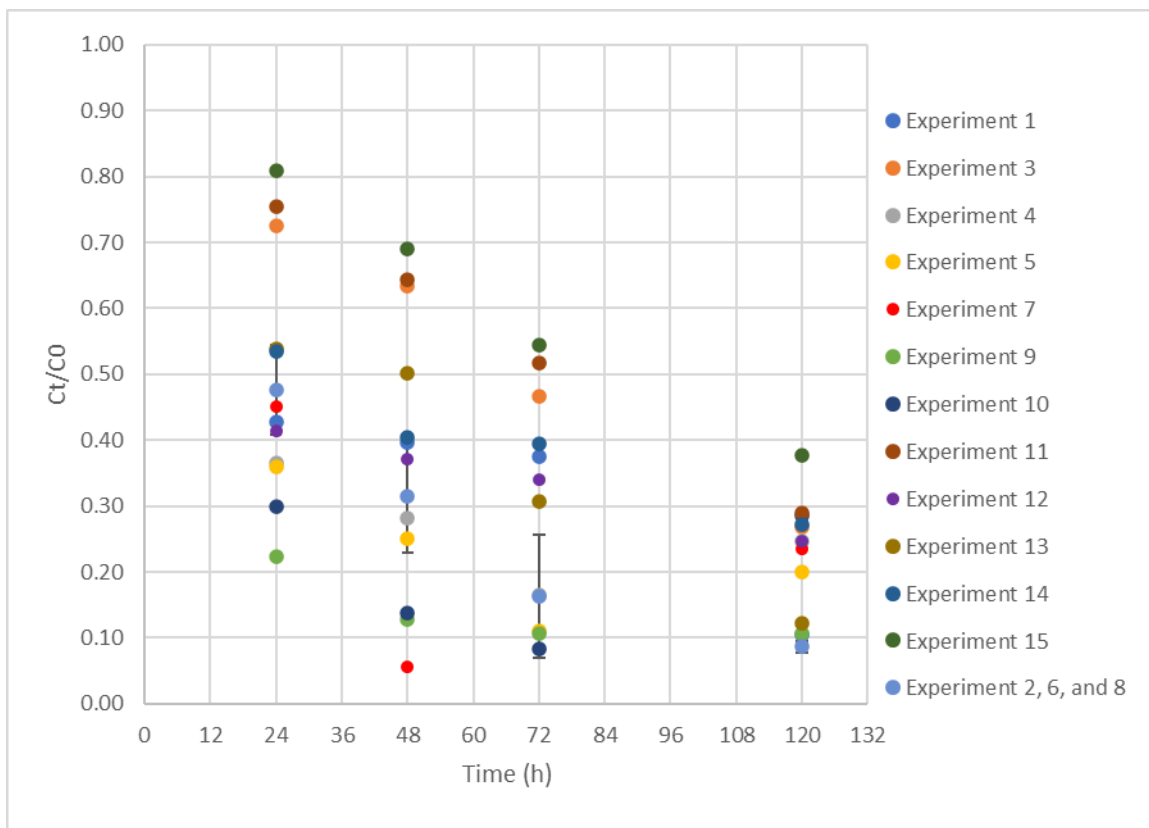


Figure 8 - Normalized concentrations ( $C_t/C_0$ ) for Gd of all 15 experiments for 120 h. The error bars are standard deviation between replicates.

### 3.3.2 Parameters for the optimized contaminants removal conditions

The removal percentages obtained in the 15 experiments were gathered and used to derive theoretical equations that identify which variables significantly affect the removal process and in what way. The variables could affect negatively with its increase leading to a lower removal, or they could impact the removal in a positive way, increasing this process the higher the parameter value. All the equations for the removal of Hg, La, and Gd at 48 h and 72 h are present in **Table 6**. These two times were chosen since most of the removal occurs between 0 and 48 h, and the removals is near the equilibrium after 72 h. Using the Analysis of Variance (ANOVA) it was possible to verify which parameters were significant ( $p$ -value lower than 0.05), and, finally, these significant parameters were used to obtain the equations in **Table 6**. The equations using ANOVA were created regarding the linear and quadratic influences and the different ways the variants interact between them that affect the removal process. The proximity

between the coefficient of determination ( $R^2$ ) and the adjusted value (adjusted  $R^2$ ) indicate the validity of the adjust done by the software.

Table 6 – Removal (%) equations regarding actual factors and their respective coefficient of determination and the adjusted coefficient of determination. Negatively and positively impact factors are represented by the colors red and green, respectively. All the equations are presented at the 48 and 72 h for each element, respectively. The proximity between  $R^2$  and adjusted  $R^2$  indicate the validity of the adjust done.

Element	Equation using actual factors	$R^2$	Adjusted $R^2$	Figure
Mercury	Removal (%) = $87.67190 - 0.487554 \times \text{Concentration} + 0.019165 \times \text{Algae mass} \times \text{Salinity} + 0.011359 \times \text{Concentration}^2$	0.5603	0.4404	9
	Removal (%) = $77.23249 + 0.148779 \times \text{Concentration} + 0.409324 \times \text{Salinity} - 0.011501 \times \text{Algae mass} \times \text{Salinity}$	0.5581	0.4376	10
Lanthanum	Removal (%) = $15.12931 + 14.46110 \times \text{Algae mass}$	0.6088	0.5787	11
	Removal (%) = $13.39499 - 0.037913 \times \text{Concentration} + 14.82551 \times \text{Algae mass} + 2.78055 \times \text{Salinity} + 0.003441 \times \text{Concentration} \times \text{Salinity} - 0.000056 \text{Concentration}^2 - 0.093351 \text{Salinity}^2$	0.9307	0.8788	12
Gadolinium	Removal (%) = $29.76700 - 0.018597 \times \text{Concentration} + 14.72947 \times \text{Algae mass}$	0.8544	0.8301	13 (A)
	Removal (%) = $36.77330 + 12.17857 \times \text{Algae mass}$	0.6949	0.6714	13 (B)

In **Figure 9**, there are the 3D responses surfaces regarding Hg for 48 h, presenting the relations between all the parameters and, in **Figure 10** for 72 h obtained through the equations calculated (present in **Table 6**).

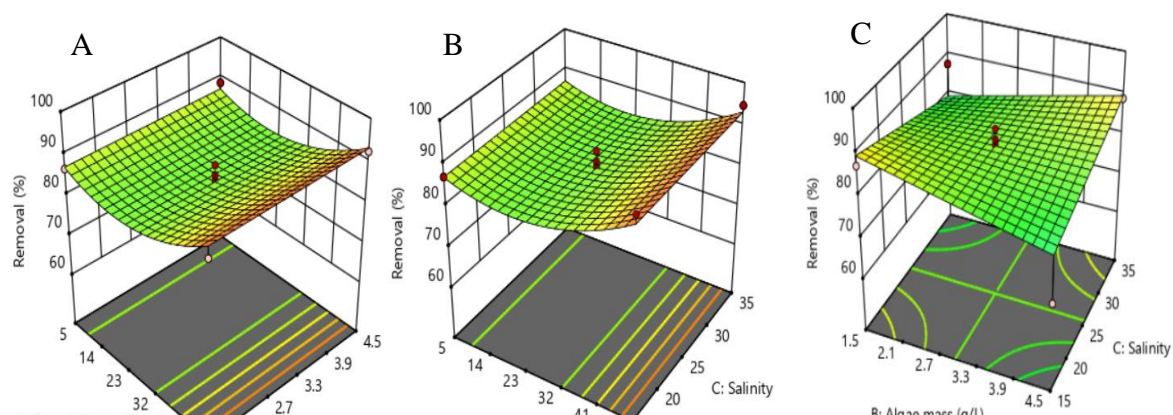
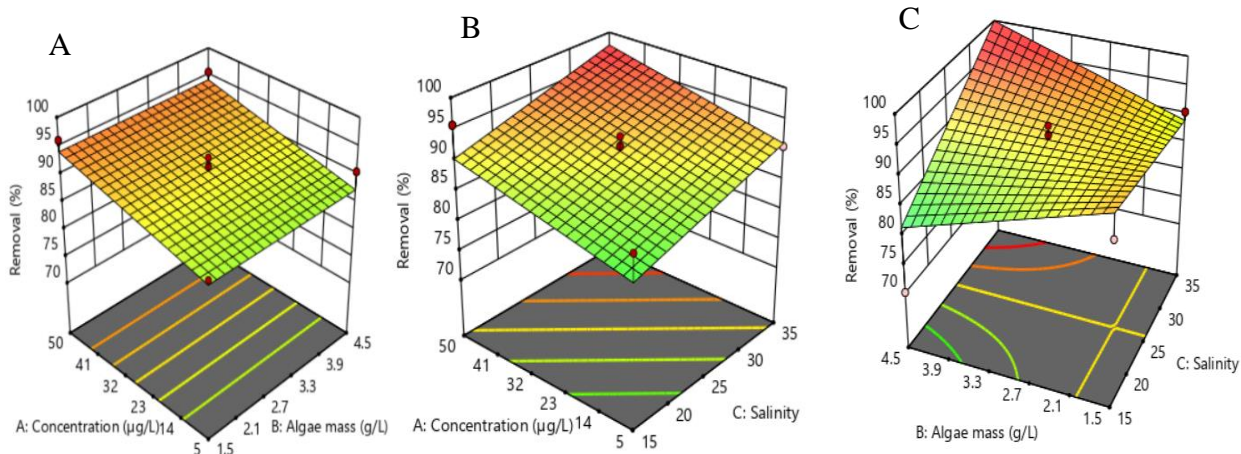


Figure 9 - 3D surface graphs at 48 h showing the different interactions effect on the Hg removal percentages. A) The interactions between concentration and algae mass, B) the interaction between concentration and salinity, C) the interaction between algae mass and salinity. The red dots represent the experimental data from the assays, being the removal percentages ranged from 0 % (light green) and 100 (dark red).



It is possible to observe in **Figure 9** that all the variables affected in some way the removal rates. For instance, the higher the concentration, the greater the removal percentages, having a positive impact. Another thing worth mentioning is that the macroalgae mass had an effect in junction with salinity (positive impact) although it is very small in comparison to the initial concentration of Hg. Regarding **Figure 10**, the variables change, and salinity has the highest impact on the process. The higher the salinity the higher the removal at 72 h, with concentration following the same trend but at a lower impact. The junction of the impact of macroalgae mass and salinity follow the opposite trend comparing to 48 h, having a negative impact on the removal percentages.

The analysis of RSM data in Design Expert also allowed to obtain the optimal conditions to maximize the removal of Hg. In the case of the 48 h, it is possible to achieve a maximum of 93 % with a concentration of 50 µg/L, an algae mass of 3 g/L, and a salinity of 25. At 72 h, using a concentration of 43.3 µg/L, a biomass of 4.4 g/L, and a salinity of 33, it is achieved a removal of virtually 100 %, according to the model.

The next two figures, **Figure 11** and **Figure 12**, are for La at 48 h and 72 h, respectively.

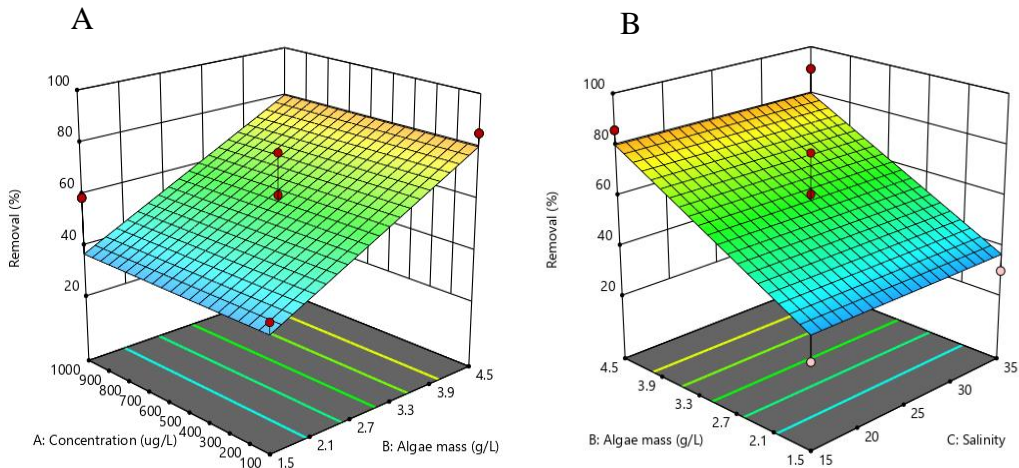


Figure 11 - 3D surface graphs at 48 h showing the different interactions effect on the La removal percentages. A) The interactions between concentration and algae mass, B) the interaction between algae mass and salinity. The red dots represent the experimental data from the assays, being the removal percentages ranged from 0 % (light green) and 100 (dark red).

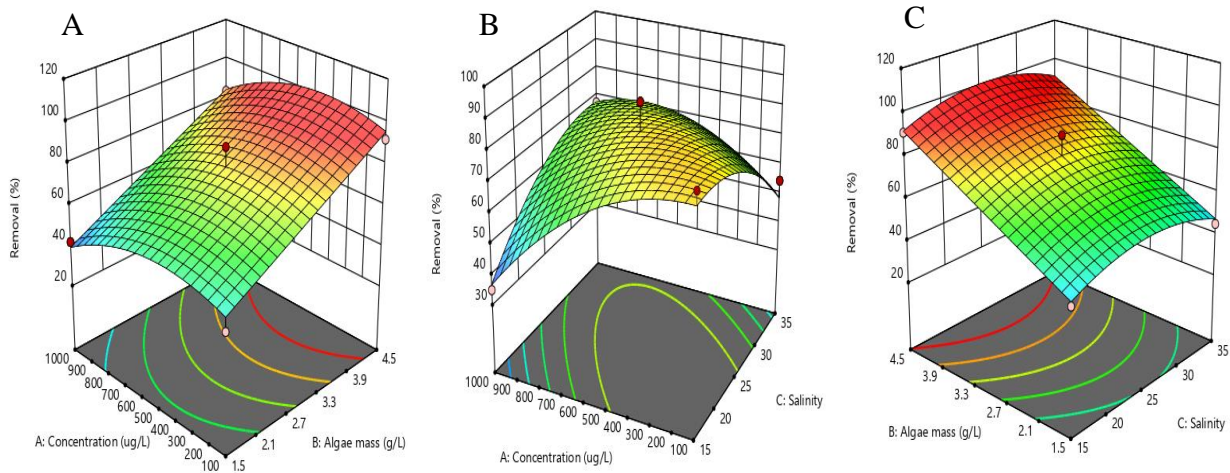


Figure 12 - 3D surface graphs at the 72h showing the different interactions effect on the La removal percentages. A) The interactions between concentration and algae mass, B) the interaction between concentration and salinity, C) the interaction between algae mass and salinity. The red dots represent the experimental data from the assays, being the removal percentages ranged from 0 % (light green) and 100 (dark red).

In **Figure 11**, it is only shown the correlations of concentration and salinity towards the mass of the macroalgae due to this last parameter being the only one to affect the removal percentage, positively. In the case of **Figure 12**, all the parameters affect the removal of La in various ways. The most impactful variant is still macroalgae mass, and then salinity, and finally a junction of concentration plus salinity, all these in a positive way. Regarding the negatively

affecting parameters, these are concentration, concentration squared, and salinity squared. According to the model created using the software, the optimal conditions to maximize the La removal would be using concentration of 550  $\mu\text{g/L}$ , algae mass of 4.5  $\text{g/L}$ , and salinity 25, reaching a removal of 80 % in 48 h. After 72 h the values for optimal removal are for concentration 439  $\mu\text{g/L}$ , macroalgae mass 4.5  $\text{g/L}$ , and salinity 23, being possible to reach a theoretical removal of 102 %.

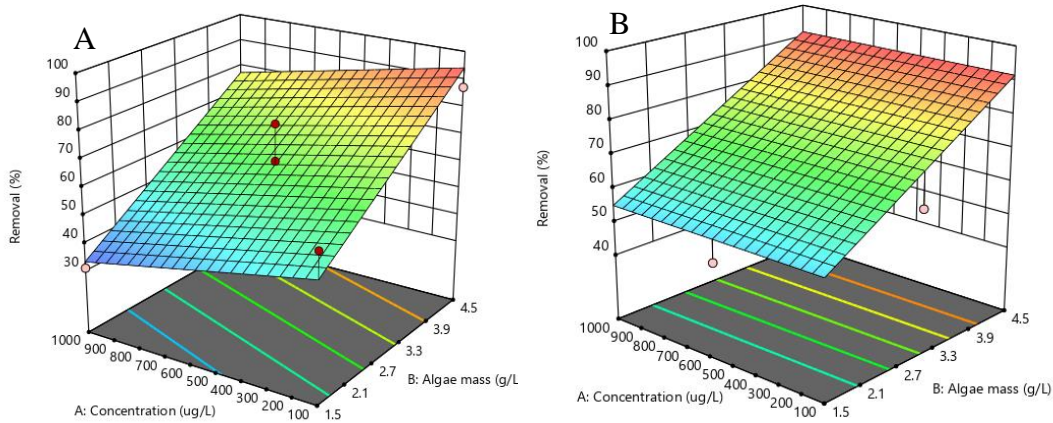


Figure 13 - 3D surface graphs showing the different interactions effect of the Gd removal percentages. A) The interactions between concentration and algae mass at 48 h, B) the interactions between concentration and algae mass at 72 h. The red dots represent the experimental data from the assays, being the removal percentages ranged from 0 % (light green) and 100 (dark red).

In **Figure 13**, it is present the 3D graphs for 48 h (A) and 72 h (B) for Gd. This is an exception, both illustrated in the same frame due to their interactions with the removal percentage being only impacted by macroalgae mass positively for both cases, and concentration negatively exclusively for the 48 h. At the earlier point in time, the optimal conditions of the highest removal percentages are 550  $\mu\text{g/L}$  of Gd, 4.5  $\text{g/L}$  of macroalgae mass, and 25 of salinity. These conditions lead to a removal of 94 %, according to the theoretical model. In the case of 72 h, the variants that can get the best results have the same values as the previous case, but with a removal percentage a little different, 92 %.

Throughout the optimization process, it was noticeable that all the factors affected in some way almost all the elements tested throughout the different experiments. First off, salinity showed a role in three of the six equations presented, in both Hg equations and in the equation regarding La at 72 h. It had a positive impact for Hg removal, having a higher impact in the removal of the contaminant at 72 h than at 48 h, where its impact was conjugated with the

macroalgae mass. This might seem contrary to what it is believed, where the higher the salinity, the higher the ions competition for the active sites, and thus lowering the removal of the desired contaminant, but in this case that does not happen. It would be also expected the formation of a complex between  $\text{Hg}^{2+}$  and  $\text{Cl}^-$  ions, which would lead to a decrease in adsorption by the biosorbent (Fabre et al., 2019). However, there is the possibility that the increase in salinity led to the increase in ionic force and the gradient between the solution and the macroalgae surface. In an attempt to decrease this gradient, the algae, having Hg high affinity towards specific functional groups present, sorbs this element to try to equalize the differences between the mixture solution and the algae. After 72 h, most of the Hg has been removed, leading to the sorption of other element, La, in the same pursue to equalize the differences previously said regarding Hg. This hypothesis is in line to what is visible in the surface graphs and the equations obtained, being known that the algae osmoregulation systems involves the capture of ions and making use of them as said by Henriques, (2014). It is also known that *U. lactuca* is a marine alga, being its preferable ecosystem salt water (seawater) as pointed out by Bews et al., (2021), stating that the decrease in salinity had negative effects on the algae.

The most prominent role was undoubtedly macroalgae mass, being present in all removal equations obtained from the software. In some situations, it was the only variant able to affect the removal. It was a crucial factor, which is in concordance to what was expected, by increasing the biosorbent mass, it is also increased the number of active sites available for the contaminant to attach too and, because the sorbent used in this case in a living algae, there is also more biomass leading to a higher bioaccumulation of the contaminant into the cells. This positive impact on the removal was also presented by Fabre et al. (2019), where it was studied the removal of Hg using *Eucalyptus globulus* bark in different dosages. What was found out was that the higher the mass of the sorbent, the higher the removal until a certain point, where it is theorized the formation of agglomerates preventing the access to certain functional groups related to the sorption of Hg. In the study of Henriques et al., (2019b) with different dosages of *U. lactuca* biomass, it was also reported a higher removal when comparing 6.0 to 1.5 g/L of macroalgae mass. The differences in this article were on all elements tested, being the more noticeable examples for Mn (from 0.17 to 0.79) and for Cr (from 0.23 to 0.77).



Regarding the initial concentration, it was a significant variable in four out of six cases in this case study. Regarding Hg, it seems that the higher the concentration the higher the removal of the element, but this is not what was observable when analyzing La and Gd. Regarding REE, the effects of concentration are on distinct times, for La at 72 h, and for Gd at 48 h of contact time and affects the removals of these two elements. It is known that the greatest concentration potentiates the highest removal percentages, like in the case of Hg in this experiment. In the case of La at 72 h, it seems that when salinity increases with concentration, the removal does so too until reaching a plateau. This plateau is the optimal conditions for these two parameters, concentration of 439  $\mu\text{g/L}$  and salinity 23. After this optimal point, the removal percentages drop with the increase of both concentration and salinity. Regarding Gd after 48 h, the concentration negatively affects the removal. This effect was also noticeable by Henriques et al. (2017b) regarding Cadmium (Cd), where the higher the initial concentration the lower the removal percentages. The initial concentration can be crucial for elements with a high affinity, like Hg, but having the opposite effect for elements that are not preferable by the algae. This decrease in removal efficiency due to a higher initial metal concentration can be explained by, later in the process, when bioaccumulation occurs, the surface of the algae is “full” of contaminants and it needs time to do the transportation from the surface to the interior of the cells and, it is easier to remove a lower concentration in solution than a much higher concentration. Although it is important initially as a driving force, as the process moves to the later steps, the lower quantity of elements in solution is beneficial, being more easily removed than when the quantity is many times higher.

Considering all the results in the present study, the objective of optimizing the process was achieved using *Ulva lactuca* to remove Hg, La, and Gd from matrices under various realistic concentrations of these contaminants, including the maximum permitted in the discharge for Hg. Results also provided some information about the conditions to maximize the removal of two high-valued REEs, opening the possibilities to reusing/recycle these elements in the future.

### 3.4 Comparative analysis with other studies

In order to test the reliability of the process and the effectiveness of the technique used it is imperative to compare it with other methodologies, regarding the biosorption and bioaccumulation, gaining these methods popularity in the last years.

For Hg, in the assays performed under the design of experiment conditions, it was observed a removal percentage that varies from 44 to 93 % after 24 h and at 120 h the values varied from 88 and 99 %. In a study by Fabre et al. (2020b), the ability of banana peels to sorb Hg was evaluated for a concentration of 50  $\mu\text{g/L}$  and after 24 h of contact time, there was a removal higher than 93 % in saline conditions and of 83 % using tap water. In the mentioned study, at 72 h of contact time, the removal percentages varied between 73 and 91 %. Despite not being the same exact conditions as the ones in present study, it can help to understand the potential of this technology. In the following **Table 7**, it is presented a compilation of different sorbents and their respective removal percentages for different contact times. Analyzing the following table, it is notable a trend, where the initial concentration of Hg is most of the times 50  $\mu\text{g/L}$  that is the mark set by the UE regarding discharges. It is also important to study higher Hg concentrations because there are some cases, such as in Southern Germany, where it was recorded values of Hg of 230  $\mu\text{g/L}$  in the groundwater (Du et al., 2015). Regarding the removal rates, it seems that the macroalgae *U. lactuca* has higher removal percentages than other algae, nutshells, and banana peels.

Table 7 - Removal (%) of Hg in different studies and sorbents.

Sorbent used	Initial concentration ( $\mu\text{g/L}$ )	Removal after 24 h (%) (mean)	Removal after the full process (%) (mean)	Highest removal recorded (%)	Author
<i>Ulva lactuca</i>	5	78	92	97	Present study
	27.5	84	94	99	
	50	87	96	98	
Banana peels	50	( $\pm$ )90	( $\pm$ )92	98	(Fabre et al., 2020b)
<i>Ulva lactuca</i>	10	( $\pm$ )76	( $\pm$ )99	( $\pm$ )99	(Henriques et al., 2015)
	50	70	( $\pm$ )98	( $\pm$ )98	
<i>Gracilaria gracilis</i>	10	( $\pm$ )64	( $\pm$ )94	( $\pm$ )94	
	50	58	( $\pm$ )97	( $\pm$ )97	
<i>Fucus vesiculosus</i>	10	( $\pm$ )59	( $\pm$ )96	( $\pm$ )96	
	50	38	( $\pm$ )88	( $\pm$ )88	
<i>Fucus vesiculosus</i>	50	( $\pm$ )45	97	97	(Henriques et al., 2017a)
<i>Ulva lactuca</i>	50	( $\pm$ )62 - 72	96 - 100	100	(Henriques et al., 2017b)
Peanut shell	50	( $\pm$ )80	88	88	(Dias et al., 2021)
Almond shell	50	( $\pm$ )70	68	68	
Walnut shell	50	( $\pm$ )76	74	74	
Hazelnut shell	50	( $\pm$ )75	78	78	
Pistachio shell	50	( $\pm$ )75	72	72	
Soybean hulls	20	94	96	96	(Rizzuti et al., 2015)
Rice hulls	20	90	96	96	

The values with a "( $\pm$ )" are estimated from the graphs present in the article cited.

The mean value was obtained using the different removal percentages of the studies for distinct conditions.

Studies shown were chosen based on the initial Hg concentration and its similarity to the one tested in this work.

The removal values were rounded to have no decimal cases.

It is important to clarify that the reported trials were not done in the same conditions (different sorbent dosages, temperature, pH, ...), so these comparisons are not absolute and just serve as a reference to evaluate the work performed. When comparing the optimal removal (for Hg concentration of 43  $\mu\text{g/L}$ , salinity of 33, and biomass of 4.4 g/L) in the present study (100 % at 72 h), some other studies approach this value, but others are lacking, only achieving between 68 and 78 %.

For La, the removal was lower, with values between 15 to 75 % after 24 h. At 120 h these values increase to 61 to 92 %. In terms of comparative analysis with other studies, it is more complicated, because the removal of La using biosorption, especially in the presence of other contaminants (in mixture), is a new topic of research. The same can be said for Gd. One study on a multi-element solution for the biosorption of La was performed by Jacinto et al., (2018), where the concentration of La tested was 500 µg/L in a mixture with other rare earth elements using the algae *Gracilaria gracilis*, and obtaining a removal of 70 % at 48 h. The study of Fabre et al., (2020a) examined the removal of 139 µg/L of La, achieving removals between 28 and 68 %, depending on the algae type tested (with the best performer being *U. lactuca* and the worst performance belonging to *Fucus spiralis*) (Table 8).

Table 8 - Removal (%) of La in different studies and sorbents.

Sorbent used	Initial concentration (µg/L)	Removal after 24 h (%) (mean)	Removal after the full process (%) (mean)	Highest removal recorded (%)	Author
<i>Ulva lactuca</i>	100	55	87	92	Present study
	550	44	80	92	
	1000	37	72	86	
<i>Gracilaria gracilis</i>	500	(±)50	70	70	(Jacinto et al., 2018)
<i>Ulva lactuca</i>	139 (1µmol/L)	-----	68	68	(Fabre et al., 2020a)
<i>Gracilaria sp.</i>		-----	61	61	
<i>Ulva intestinalis</i>		-----	63	63	
<i>Fucus spiralis</i>		-----	28	28	
<i>Fucus vesiculosus</i>		-----	41	41	
<i>Osmundea pinnatifida</i>		-----	33	33	

The values with a (±) are estimated from the graphs present in the article cited.

The mean value was obtained using different removal percentages of the studies for distinct conditions.

Studies shown were chosen based on the initial Hg concentration and its similarity to the one tested in this work.

The removal values were rounded to have no decimal cases.

Other studies regarding the removal of La, used a higher concentrations of the element, such as 10 and 200 mmol/L (Iannicelli-Zubiani et al., 2018), 60 and 160 mg/L (Kusrini et al., 2018), 50 mg/L (Gabor et al., 2017), 5 mg/L (Marwani et al., 2017). These concentrations are

environmental unrealistic, like in the case of Finland study, where La values range from 0.18 and 900  $\mu\text{g/L}$  (Astrom, 2001). In this work, with the optimal conditions (La concentration of 439  $\mu\text{g/L}$ , salinity of 23, and biomass of 4.5  $\text{g/L}$ ) it was possible to remove 100 % of La, which is a higher value than the ones of the cited articles, that showed values between 33 and 70 %.

For Gd at 24 h, the removal ranged from 19 to 78 %. At 120 h the removals reached values between 62 and 92 %. The removal of Gd is slightly higher than the one regarding La.

Table 9 - Removal (%) of Gd in different studies and sorbents.

<b>Sorbent used</b>	<b>Initial concentration (<math>\mu\text{g/L}</math>)</b>	<b>Removal after 24 h (%) (mean)</b>	<b>Removal after the full process (%) (mean)</b>	<b>Highest removal recorded (%)</b>	<b>Author</b>
<i>Ulva lactuca</i>	100	61	83	90	Present study
	550	48	81	92	
	1000	47	75	88	
<i>Ulva lactuca</i>	157 ( $1\mu\text{mol/L}$ )	-----	72	72	(Fabre et al., 2020a)
<i>Gracilaria sp.</i>		-----	69	69	
<i>Ulva intestinalis</i>		-----	67	67	
<i>Fucus spiralis</i>		-----	32	32	
<i>Fucus vesiculosus</i>		-----	38	38	
<i>Osmundea pinnatifida</i>		-----	38	38	

The values with a ( $\pm$ ) are estimated from the graphs present in the article cited.

The mean value was obtained using different removal percentages of the studies for distinct conditions.

Studies shown were chosen based on the initial Hg concentration and its similarity to the one tested in this work.

The removal values were rounded to have no decimal cases.

Studies with Gd removal usually worked with high and unrealistic concentrations: use of rice husk to remove 100  $\text{mg/L}$  of Gd (Gad et al., 2017), chitosan films to remove 50  $\text{mg/L}$  (Zheng et al., 2017), and using ionic columns to remove 400  $\text{mg/L}$  (Hamed et al., 2016), which is 400 times greater than the maximum concentration used on this study. When comparing these values with ones present in the environment, like between 200 and 1100  $\mu\text{g/L}$  reported by Rogowska et al. (2017) and between 0.002 and 207  $\mu\text{g/L}$  reported by Astrom (2001), it is clear

that most studies used unrealistic concentrations. At optimal conditions (Gd concentration of 550 µg/L, salinity of 25, and biomass of 4.5 g/L) and at 48 h, the removal percentage was 94 %.

### 3.5 Impacts on macroalgae due to exposure to Hg, La, and Gd

The physiological condition of the macroalgae was evaluated, mainly through direct observation, after the exposure to the contaminants. After 120 h, some negative effects on the macroalgae were detected, such as some discoloration and tenderness (fragility) as shown in **Figure 14**.



Figure 14 - Noticeable effects on the macroalgae after 120 h of exposure.

This could be due to the duration of the exposure, with these effects only being noticeable after 120 h. This fact can be overlooked in a more practical application because most algae had optimal removal percentages at 72 h, and just reaching a plateau at 120 h. This also validates the point of, in some cases, at 120 h having negative removal percentages in comparison to the previous sampling time. Because of this, it is possible to apply the process with reliability by finishing the assay sooner to avoid these issues and still have good removal percentages. This same effect was present on the control (algae with seawater and no contamination), meaning that this effect could be due to biotic and abiotic factors who weren't controlled and/or identified in the present study.

The amount of chlorophyll present in the macroalgae before and after the procedure was also measured to evaluate if any of the elements had a negative effect on it. The expected result if the toxicity potentiated the algae to have a lower concentration of chlorophyll (Čypaitė et al., 2014; Siedlewicz et al., 2020), but such that did not happen, as a matter in fact, the opposite was verified, the increase in the amount of chlorophyll. It seems the algae suffered in some degree with the contact of toxic elements, but not affecting the chlorophyll quantity. This was reported by Řezanka et al. (2016), who tested the quantity of chlorophyll “a”, “b”, and the ratio between a/b in a green algae (*Desmodesmus quadricauda*) in the presence of 1 µmol/L of La (139 µg/L), Gd (157 µg/L), Nd (144 µg/L), and Ce (140 µg/L). It was reported that there was an increase in chlorophyll “b” in all cases, having chlorophyll “a” remained unchanged, leading to a decrease in the ratio. This is something expected and previously reported when the environment changes and/or in the presence of trace metals, but could be also due to the fact that there is a possible stimulation of photosystem membrane by low concentrations of REE, as showed by Kruk et al., (2003) when using Eu and Dy. In this study, it is not known the ratio, but the relative values of the quantity of chlorophyll present are in concordance with the article cited, having an increase when in contact with elements such as La and Gd. The values of these can be found in **Table 10**.

Table 10 - Quantity of chlorophyll (qualitative values) for macroalgae before the trial, after the trial, and their difference.

<b>Experiment</b>	<b>Chlorophyll before the trial (mean)</b>	<b>Chlorophyll after the trial (mean)</b>	<b>Chlorophyll difference (after – before)</b>
<b>1</b>	8.1	12.4	4.3
<b>2</b>	8.0	11.5	3.5
<b>3</b>	8.9	11.8	2.9
<b>4</b>	7.4	13.2	5.8
<b>5</b>	7.5	10.2	2.7
<b>6</b>	8.7	17.2	8.5
<b>7</b>	11.7	19.4	7.7
<b>8</b>	10.5	17.0	6.5
<b>9</b>	8.1	11.3	3.2
<b>10</b>	12.4	15.8	3.4
<b>11</b>	7.8	8.5	0.7
<b>12</b>	9.8	8.3	-1.5
<b>13</b>	8.4	13.8	5.4
<b>14</b>	10.0	15.4	5.4
<b>15</b>	13.2	17.7	4.5

## 4. Conclusions

Water systems with quality are getting scarcer each year, due to the increase in population, leading to anthropogenic contamination of aquatic bodies. The remediation of the contaminated aquatic ecosystems is imperative, once it can lead to serious consequences. It is known that aquatic systems are stressed due to high contamination with not only classic but also emerging contaminants. Conventional removal methodologies can fall a bit short of the main goal and the challenge of the present work was to evaluate a cheap, simple, effective, and sustainable methodology to treat contaminated water. Biosorption and bioaccumulation proved to be promising methodologies to follow-through regarding the recovery of contaminated aquatic ecosystems, whilst still having room to evolve.

The removal of Hg, La, and Gd, in mono and multi-element solutions using *Ulva lactuca* showed promising results, being this biosorbent able to remove large quantities of these three elements from contaminated water. This validates the various claims about its potential as a promising technology for the bioremediation of waters.

The optimization of the process using the Response Surface Methodology (RSM) was a novelty in literature, and its use, has allowed to gather a large amount of information with a low set of experiences. The parameter optimization process regarding the salinity, biosorbent dosage, and contaminant initial concentration shows great findings, proving that this technology is not only extremely flexible, but also as still room to improve and develop. The novelty of this work was in focusing on complex matrices, which is scarce in literature, through multi-contaminated solutions and studying the optimization of the process.

A step following this one, could be the evaluation of the optimal conditions in real matrices to further increase the validity of the technology. It still would be important to study if it is possible to recover these elements in order to be recycled and reused by the industry, leading to a circular economy.



## 5. References

- 1 - Water Scarcity. <https://www.worldwildlife.org/threats/water-scarcity> - last accessed on 02/10/20.
  - 2 - Water Scarcity. <https://www.unwater.org/water-facts/scarcity/>. – last accessed on 03/10/20.
  - 3 - Water, Sanitation and Hygiene. <https://www.unicef.org/wash/>. – last accessed on 14/11/20.
  - 4 - Water Recycling. <https://www.watereducation.org/aquapedia/water-recycling>. - last accessed on 05/10/20.
  - 5 - UNICEF and the Sustainable Development Goals. <https://www.unicef.org/sdgs>. - last accessed on 14/11/20
- Agarwal, A., Upadhyay, U., Sreedhar, I., Singh, S.A., Patel, C.M., 2020. A review on valorization of biomass in heavy metal removal from wastewater. *J. Water Process Eng.* 38, 101602, 1–25.
- Aharchaou, I., Beaubien, C., Campbell, P.G.C., Fortin, C., 2020. Lanthanum and Cerium Toxicity to the Freshwater Green Alga *Chlorella fusca*: Applicability of the Biotic Ligand Model. *Environ. Toxicol. Chem.* 39, 996–1005.
- Armin, F., Talpur, F.N., Balouch, A., Chandio, Z.A., Surhio, M.A., Afridi, H.I., 2016. Biosorption of Mercury(II) from Aqueous Solution by Fungal Biomass *Pleurotus eryngii*: Isotherm, Kinetic, and Thermodynamic Studies. *Environ. Prog. Sustain. Energy* 1–9.
- Astrom, M., 2001. Abundance and fractionation patterns of rare earth elements in streams affected by acid sulphate soils 249–258.
- Ayangbenro, A.S., Babalola, O.O., 2017. A new strategy for heavy metal polluted environments: A review of microbial biosorbents. *Int. J. Environ. Res. Public Health* 14, 94, 1–16.

- Bendakovská, L., Krejčová, A., Weidlich, T., 2019. Sorption and biosorption of Gd-based contrast agents in the water environment. *Chem. Pap.* 73, 2995–3003.
- Bews, E., Booher, L., Polizzi, T., Long, C., Kim, J.H., Edwards, M.S., 2021. Effects of salinity and nutrients on metabolism and growth of *Ulva lactuca*: Implications for bioremediation of coastal watersheds. *Mar. Pollut. Bull.* 166, 112199.
- Bratby, J., 1980. *Coagulation and Flocculation and With an emphasis on water and wastewater treatment.* Uplands Press Ltd.
- Carpenter, D., Boutin, C., Allison, J.E., Parsons, J.L., Ellis, D.M., 2015. Uptake and Effects of Six Rare Earth Elements (REEs) on Selected Native and Crop Species Growing in Contaminated Soils. *PLoS One* 10, e0129936, 1–21.
- Corrêa, F. do N., Luna, A.S., da Costa, A.C.A., 2017. Kinetics and equilibrium of lanthanum biosorption by free and immobilized microalgal cells. *Adsorpt. Sci. Technol.* 35, 137–152.
- Crawford, C.B., Quinn, B., 2017. The interactions of microplastics and chemical pollutants, in: *Microplastic Pollutants.* Elsevier Science, pp. 131–157.
- Čypaitė, A., Žaltauskaitė, J., Vencloviene, J., 2014. Assessment of chlorophyll-a, chlorophyll-b and growth rate in freshwater green algae *Pseudokirchneriella subcapitata* exposed to cadmium and copper. 9th Int. Conf. Environ. Eng. ICEE 2014.
- Dahman, Y., 2017. Nanopolymers, in: *Nanotechnology and Functional Materials for Engineers.* Elsevier, pp. 121–144.
- Das, N., Das, D., 2013. Recovery of rare earth metals through biosorption: An overview. *J. Rare Earths* 31, 933–943.
- Di Caprio, F., Altamari, P., Zanni, E., Uccelletti, D., Toro, L., Pagnanelli, F., 2016. Lanthanum biosorption by different *Saccharomyces cerevisiae* strains. *Chem. Eng. Trans.* 49, 37–42.

- Dias, M., Pinto, J., Henriques, B., Figueira, P., Fabre, E., Tavares, D., Vale, C., Pereira, E., 2021. Nutshells as efficient biosorbents to remove cadmium, lead, and mercury from contaminated solutions. *Int. J. Environ. Res. Public Health* 18, 1–17.
- Du, M., Wei, D., Tan, Z., Lin, A., Du, Y., 2015. Predicted no-effect concentrations for mercury species and ecological risk assessment for mercury pollution in aquatic environment. *J. Environ. Sci. (China)* 28, 74–80.
- El-Sayed, M., Nada, A.A., 2017. Polyethylenimine – functionalized amorphous carbon fabricated from oil palm leaves as a novel adsorbent for Cr (VI) and Pb (II) from aqueous solution. *J. Water Process Eng.* 16, 296–308.
- Entezari, M.H., Tahmasbi, M., 2009. Water softening by combination of ultrasound and ion exchange. *Ultrason. - Sonochemistry* 16, 356–360.
- Fabre, E., Dias, M., Costa, M., Henriques, B., Vale, C., Lopes, C.B., Pinheiro-Torres, J., Silva, C.M., Pereira, E., 2020a. Negligible effect of potentially toxic elements and rare earth elements on mercury removal from contaminated waters by green, brown and red living marine macroalgae. *Sci. Total Environ.* 724, 138133, 1–11.
- Fabre, E., Lopes, C.B., Vale, C., Pereira, E., Silva, C.M., 2020b. Valuation of banana peels as an effective biosorbent for mercury removal under low environmental concentrations. *Sci. Total Environ.* 709, 135883, 1–9.
- Fabre, E., Vale, C., Pereira, E., Silva, C.M., 2021. Sustainable Water Treatment: Use of Agricultural and Industrial Wastes to Remove Mercury by Biosorption. *Water, Air, Soil Pollut.*
- Fabre, E., Vale, C., Pereira, E., Silva, C.M., 2019. Experimental Measurement and Modeling of Hg (II) Removal from Aqueous Solutions Using *Eucalyptus globulus* Bark: Effect of pH, Salinity and Biosorbent Dosage. *Int. J. Mol. Sci.* 20, 5973, 1–17.

- Fomina, M., Gadd, G.M., 2014. Biosorption: current perspectives on concept, definition and application. *Bioresour. Technol.* 160, 3–14.
- Fu, F., Wang, Q., 2011. Removal of heavy metal ions from wastewaters: A review. *J. Environ. Manage.* 92, 407–418.
- Gabor, A., Davidescu, C.M., Negrea, A., Ciopec, M., Grozav, I., Negrea, P., Duteanu, N., 2017. Optimizing the lanthanum adsorption process onto chemically modified biomaterials using factorial and response surface design. *J. Environ. Manage.* 204, 839–844.
- Gad, H.M.H., Hamed, M.M., Abo Eldahab, H.M.M., Moustafa, M.E., El-Reefy, S.A., 2017. Radiation-induced grafting copolymerization of resin onto the surface of silica extracted from rice husk ash for adsorption of gadolinium. *J. Mol. Liq.* 231, 45–55.
- Gelardi, G., Flatt, R.J., 2016. Working mechanisms of water reducers and superplasticizers, in: Aïtcin, P., Flatt, R.J. (Eds.), *Science and Technology of Concrete Admixtures*. Woodhead Publishing, pp. 257–278.
- Gisi, S.D., Lofrano, G., Grassi, M., Notarnicola, M., 2016. Characteristics and adsorption capacities of low-cost sorbents for wastewater treatment: A review. *Sustain. Mater. Technol.* 9, 10–40.
- Gupta, N.K., Gupta, A., Ramteke, P., Sahoo, H., Sengupta, A., 2019. Biosorption-a green method for the preconcentration of rare earth elements (REEs) from waste solutions: A review. *J. Mol. Liq.* 274, 148–164.
- Gupta, V.K., Nayak, A., Agarwal, S., 2015. Bioadsorbents for remediation of heavy metals: Current status and their future prospects. *Environ. Eng. Res.* 20, 1–18.
- Hamed, M.M., Rizk, S.E., Nayl, A.A., 2016. Adsorption kinetics and modeling of gadolinium and cobalt ions sorption by an ion-exchange resin. *Part. Sci. Technol.* 34, 716–724.
- Henriques, B., 2014. Metais em plantas e algas: acumulação e potencial aplicação em processos

de remediação Metais em plantas e algas: acumulação e potencial aplicação em processos de remediação.

Henriques, B., Coppola, F., Monteiro, R., Pinto, J., Viana, T., Pretti, C., Soares, A., Freitas, R., Pereira, E., 2019a. Toxicological assessment of anthropogenic Gadolinium in seawater: Biochemical effects in mussels *Mytilus galloprovincialis*. *Sci. Total Environ.* 664, 626–634.

Henriques, B., Lopes, C.B., Figueira, P., Rocha, L.S., Duarte, A.C., Vale, C., Pardal, M.A., Pereira, E., 2017a. Bioaccumulation of Hg, Cd and Pb by *Fucus vesiculosus* in single and multi-metal contamination scenarios and its effect on growth rate. *Chemosphere* 171, 208–222.

Henriques, B., Rocha, L.S., Lopes, C.B., Figueira, P., Duarte, A.C., Vale, C., Pardal, M.A., Pereira, E., 2017b. A macroalgae-based biotechnology for water remediation: Simultaneous removal of Cd, Pb and Hg by living *Ulva lactuca*. *J. Environ. Manage.* 191, 275–289.

Henriques, B., Rocha, L.S., Lopes, C.B., Figueira, P., Monteiro, R.J.R., Duarte, A.C., Pardal, M.A., Pereira, E., 2015. Study on bioaccumulation and biosorption of mercury by living marine macroalgae: Prospecting for a new remediation biotechnology applied to saline waters. *Chem. Eng. J.* 281, 759–770.

Henriques, B., Teixeira, A., Figueira, P., Reis, A.T., Almeida, J., Vale, C., Pereira, E., 2019b. Simultaneous removal of trace elements from contaminated waters by living *Ulva lactuca*. *Sci. Total Environ.* 652, 880–888.

Hsu, C.J., Cheng, Y.H., Huang, Y.P., Atkinson, J.D., Hsi, H.C., 2021. A novel synthesis of sulfurized magnetic biochar for aqueous Hg(II) capture as a potential method for environmental remediation in water. *Sci. Total Environ.* 784, 147240.

Iannicelli-Zubiani, E.M., Gallo Stampino, P., Cristiani, C., Dotelli, G., 2018. Enhanced

- lanthanum adsorption by amine modified activated carbon. *Chem. Eng. J.* 341, 75–82.
- Ihsanullah, Abbas, A., Al-Amer, A.M., Laoui, T., Al-Marri, M.J., Nasser, M.S., Khraisheh, M., Ali, M.A., 2016. Heavy metal removal from aqueous solution by advanced carbon nanotubes: Critical review of adsorption applications. *Sep. Purif. Technol.* 157, 141–161.
- Inyang, M.I., Gao, B., Yao, Y., Xue, Y., Zimmerman, A., Mosa, A., Pullammanappallil, P., Ok, Y.S., Cao, X., Inyang, M.I., Gao, B., Yao, Y., Xue, Y., Zimmerman, A., 2016. A review of biochar as a low-cost adsorbent for aqueous heavy metal removal. *Crit. Rev. Environ. Sci. Technol.* 46, 406–433.
- Jacinto, J., Henriques, B., Duarte, A.C., Vale, C., Pereira, E., 2018. Removal and recovery of Critical Rare Elements from contaminated waters by living *Gracilaria gracilis*. *J. Hazard. Mater.* 344, 531–538.
- Jaireth, S., Hoatson, D.M., Mieziotis, Y., 2014. Geological setting and resources of the major rare-earth-element deposits in Australia. *Ore Geol. Rev.* 62, 72–128.
- Janssen, S.E., Tate, M.T., Krabbenhoft, D.P., DeWild, J.F., Ogorek, J.M., Babiarz, C.L., Sowers, A.D., Tuttle, P.L., 2021. The influence of legacy contamination on the transport and bioaccumulation of mercury within the Mobile River Basin. *J. Hazard. Mater.* 404, 124097, 1–10.
- Jiménez, S., Micó, M.M., Arnaldos, M., Medina, F., Contreras, S., 2018. State of the art of produced water treatment. *Chemosphere* 192, 186–208.
- Kabata-Pendias, A., Mukherjee, A.B., 2007. *Trace Elements from Soil to Human*. Springer-Verlag Berlin Heidelberg.
- Kadukova, J., 2016. Surface sorption and nanoparticle production as a silver detoxification mechanism of the freshwater alga *Parachlorella kessleri*. *Bioresour. Technol.* 216, 406–413.

- Kanwar, V.S., Sharma, A., Srivastav, A.L., Rani, L., 2020. Phytoremediation of toxic metals present in soil and water environment: a critical review. *Environ. Sci. Pollut. Res.* 27, 44835–44860.
- Kazak, E.S., Kalitina, E.G., Kharitonova, N.A., Chelnokov, G.A., Elovskii, E. V., Bragin, I. V., 2018. Biosorption of Rare-Earth Elements and Yttrium by Heterotrophic Bacteria in an Aqueous Environment. *Moscow Univ. Geol. Bull.* 73, 287–294.
- Keshtkar, A.R., Moosavian, M.A., Sohbatzadeh, H., Mofras, M., 2019. La(III) and Ce(III) biosorption on sulfur functionalized marine brown algae *Cystoseira indica* by xanthation method: Response surface methodology, isotherm and kinetic study. *Groundw. Sustain. Dev.* 8, 144–155.
- Konica Minolta, 2017. Chlorophyll Meter SPAD-502 Plus 1–4.
- Kruk, J., Burda, K., Jemioła-rzemin, M., Strzalka, K., 2003. The 33 kDa Protein of Photosystem II Is a Low-Affinity Calcium- and Lanthanide-Binding Protein 14862–14867.
- Kucuker, M.A., Wiczorek, N., Kuchta, K., Coptly, N.K., 2017. Biosorption of neodymium on *Chlorella vulgaris* in aqueous solution obtained from hard disk drive magnets. *PLoS One* 2, e0175255, 1–13.
- Kumar, K.S., Dahms, H., Won, E., Lee, J., Shin, K., 2015. Microalgae – A promising tool for heavy metal remediation. *Ecotoxicol. Environ. Saf.* 113, 329–352.
- Kumar, M., Nandi, M., Pakshirajan, K., 2021. Recent advances in heavy metal recovery from wastewater by biogenic sulfide precipitation. *J. Environ. Manage.* 278, 111555, 1–14.
- Kumar, R., Mudhoo, A., Lofrano, G., Chandra, M., 2014. Biomass-derived biosorbents for metal ions sequestration: Adsorbent modification and activation methods and adsorbent regeneration. *J. Environ. Chem. Eng.* 2, 239–259.
- Kusrini, E., Wicaksono, W., Gunawan, C., Daud, N.Z.A., Usman, A., 2018. Kinetics,

mechanism, and thermodynamics of lanthanum adsorption on pectin extracted from durian rind. *J. Environ. Chem. Eng.* 6, 6580–6588.

Lerat-Hardy, A., Coynel, A., Dutruch, L., Pereto, C., Bossy, C., Gil-Diaz, T., Capdeville, M., Blanc, G., Schäfer, J., 2019. Rare Earth Element fluxes over 15 years into a major European Estuary (Garonne-Gironde, SW France): Hospital effluents as a source of increasing gadolinium anomalies. *Sci. Total Environ.* 656, 409–420.

Liem-Nguyen, V., Skjellberg, U., Björn, E., 2021. Methylmercury formation in boreal wetlands in relation to chemical speciation of mercury (II) and concentration of low molecular mass thiols. *Sci. Total Environ.* 755, 142666, 1–9.

Liu, P., Xue, Y., Ma, F., 2019. The influence of pH, contact time, ionic strength, temperature, and fullerene on the adsorption of Cu(II) onto magnetic multi-walled carbon nanotubes. *Fullerenes, Nanotub. Carbon Nanostructures* 27, 317–322.

Mansour, H., Abou El-soud, Y., 2017. Dyes bio-sorption by two marine algae and their applications on industrial effluents from Borg El-Arab region, Egypt. *Egypt. J. Bot.* 57, 49–73.

Martino, C., Bonaventura, R., Byrne, M., Roccheri, M., Matranga, V., 2016. Effects of exposure to gadolinium on the development of geographically and phylogenetically distant sea urchins species. *Mar. Environ. Res.* 128, 98–106.

Marwani, H.M., Albishri, H.M., Jalal, T.A., Soliman, E.M., 2017. Study of isotherm and kinetic models of lanthanum adsorption on activated carbon loaded with recently synthesized Schiff's base. *Arab. J. Chem.* 10, S1032–S1040.

Michalak, I., Chojnacka, K., Witek-Krowiak, A., 2013. State of the Art for the Biosorption Process — a Review. *Appl. Biochem. Biotechnol.* 170, 1389–1416.

Migaszewski, Z.M., Gałuszka, A., 2015. The Characteristics, Occurrence, and Geochemical



- Behavior of Rare Earth Elements in the Environment: A Review. *Environ. Sci. Technol.* 45, 37–41.
- Mimba, M.E., Jude, W.M., Fils, S.C.N., Numanami, N., Nforba, M.T., Ohba, T., Aka, F.T., Suh, C.E., 2020. Geochemical Behavior of REE in Stream Water and Sediments in the Gold - Bearing Lom Basin, Cameroon: Implications for Provenance and Depositional Environment. *Aquat. Geochemistry* 26, 53–70.
- Moreira, A., Henriques, B., Leite, C., Libralato, G., Pereira, E., Freitas, R., 2020. Potential impacts of lanthanum and yttrium through embryotoxicity assays with *Crassostrea gigas*. *Ecol. Indic.* 108, 105687, 1–8.
- Okenicova, L., Zemberyova, M., Procházková, S., 2016. Biosorbents for solid-phase extraction of toxic elements in waters. *Environ. Chem. Lett.* 14, 67–77.
- Oswell, N.J., Amarowicz, R., Pegg, R.B., 2019. Food and Nutritional Analysis| Fruits and Fruit Products, in: Worsfold, P., Townshend, A., Poole, C., Miró, M. (Eds.), *Encyclopedia of Analytical Science*. Elsevier, pp. 428–435.
- Pavoni, E., Crosera, M., Petranich, E., Adami, G., Faganeli, J., Covelli, S., 2020. Partitioning and mixing behaviour of trace elements at the Isonzo Soča River mouth (Gulf of Trieste, northern Adriatic Sea). *Mar. Chem.* 223, 103800, 1–14.
- Pinto, J., Costa, M., Leite, C., Borges, C., Coppola, F., Henriques, B., Monteiro, R., Russo, T., Cosmo, A.D., Soares, A.M.V.M., Polese, G., Pereira, E., Freitas, R., 2019. Ecotoxicological effects of lanthanum in *Mytilus galloprovincialis*: Biochemical and histopathological impacts. *Aquat. Toxicol.* 211, 181–192.
- Qin, C., Wang, H., Yuan, X., Xiong, T., Zhang, J., Zhang, J., 2020. Understanding structure-performance correlation of biochar materials in environmental remediation and electrochemical devices. *Chem. Eng. J.* 382, 122977, 1–23.

- Qu, X., Alvarez, P.J.J., Li, Q., 2013. Applications of nanotechnology in water and wastewater treatment. *Water Res.* 47, 3931–3946.
- Řezanka, T., Kaineder, K., Mezricky, D., Řezanka, M., Bišová, K., Zachleder, V., Vítová, M., 2016. The effect of lanthanides on photosynthesis, growth, and chlorophyll profile of the green alga *Desmodesmus quadricauda*. *Photosynth. Res.* 130, 335–346.
- Rice, K.M., Jr, E.M.W., Wu, M., Gillette, C., Blough, E.R., 2014. Environmental Mercury and Its Toxic Effects. *J. Prev. Med. Public Heal.* 47, 74–83.
- Rizzuti, A.M., Ellis, F.L., Cosme, L.W., 2015. Biosorption of mercury from dilute aqueous solutions using soybean hulls and rice hulls. *Waste and Biomass Valorization* 6, 561–568.
- Roberts, D.A., Paul, N.A., Dworjany, S.A., Bird, M.I., Nys, R., 2015. Biochar from commercially cultivated seaweed for soil amelioration. *Sci. Rep.* 5, 9665, 1–6.
- Rodriguez-Freire, L., Gonzalez-Estrella, J., Li, G., 2020. Technologies for fractionation of wastewater and resource recovery, in: *Wastewater Treatment Residues as Resources for Biorefinery Products and Energy*. Elsevier, pp. 329–354.
- Rodriguez-Narvaez, O.M., Peralta-Hernandez, J.M., Goonetilleke, A., Bandala, E.R., 2017. Treatment technologies for emerging contaminants in water: A review. *Chem. Eng. J.* 323, 361–380.
- Rodríguez, I.A., Pacheco-Castillo, N.C., Cárdenas-González, J.F., de Guadalupe Moctezuma Zarate, M., Martínez-Juárez, V.M., Rodríguez-Pérez, A., 2018. Biosorption of mercury (II) from aqueous solution onto biomass of *Aspergillus niger*. *Mex. J. Biotechnol.* 3, 15–25.
- Rogowska, J., Olkowska, E., Ratajczyk, W., Wolska, L., 2017. Gadolinium as a new emerging contaminant of aquatic environment. *Environ. Toxicol. Chem.* 37, 1523–1534.
- Salazar-Camacho, C., Salas-Moreno, M., Paternina-Uribe, R., Marrugo-Negrete, J., Díez, S., 2021. Mercury species in fish from a tropical river highly impacted by gold mining at the

- Colombian Pacific region. *Chemosphere* 264, 128478, 1–10.
- Sharma, G., Kumar, A., Sharma, S., Naushad, M., Dwivedi, R.R., AlOthman, Z.A., Mola, G.T., 2019. Novel development of nanoparticles to bimetallic nanoparticles and their composites: A review. *J. King Saud Univ. - Sci.* 31, 257–269.
- Shibaeva, T.G., Mamaev, A. V., Sherudilo, E.G., 2020. Evaluation of a SPAD-502 Plus Chlorophyll Meter to Estimate Chlorophyll Content in Leaves with Interveinal Chlorosis. *Russ. J. Plant Physiol.* 67, 690–696.
- Siedlewicz, G., Żak, A., Sharma, L., Kosakowska, A., Pazdro, K., 2020. Effects of oxytetracycline on growth and chlorophyll a fluorescence in green algae (*Chlorella vulgaris*), diatom (*Phaeodactylum tricornutum*) and cyanobacteria (*Microcystis aeruginosa* and *Nodularia spumigena*). *Oceanologia* 62, 214–225.
- Singh, R., Hankins, N.P., 2016. Introduction to Membrane Processes for Water Treatment, in: *Emerging Membrane Technology for Sustainable Water Treatment*. Elsevier Science, pp. 15–52.
- Singh, S., Kumar, V., Datta, S., Dhanjal, D.S., Sharma, K., Samuel, J., Singh, J., 2020. Current advancement and future prospect of biosorbents for bioremediation. *Sci. Total Environ.* 709, 135895, 1–24.
- Smith, L.D., Garg, U., 2017. Disorders of trace metals, in: *Biomarkers in Inborn Errors of Metabolism*. Elsevier, pp. 399–426.
- Smith, Y.R., Bhattacharyya, D., Willhard, T., Misra, M., 2016. Adsorption of aqueous rare earth elements using carbon black derived from recycled tires. *Chem. Eng. J.* 296, 102–111.
- Tan, X., Liu, Y., Zeng, G., Wang, X., Hu, X., Gu, Y., Zhongzhu, Y., 2015. Application of biochar for the removal of pollutants from aqueous solutions. *Chemosphere* 125, 70–85.
- Taylor, P., Husein, D.Z., 2013. *Desalination and Water Treatment Adsorption and removal of*

mercury ions from aqueous solution using raw and chemically modified Egyptian mandarin peel using raw and chemically modified Egyptian mandarin peel 37–41.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J., 2012. Heavy Metal Toxicity and the Environment. *Heal. Hum. Serv. Public Access* 101, 133–164.

Teodosiu, C., Gilca, A., Barjoveanu, G., Fiore, S., 2018. Emerging pollutants removal through advanced drinking water treatment: A review on processes and environmental performances assessment. *J. Clean. Prod.* 197, 1210–1221.

Tepe, N., Romero, M., Bau, M., 2014. High-technology metals as emerging contaminants: Strong increase of anthropogenic gadolinium levels in tap water of Berlin, Germany, from 2009 to 2012. *Appl. Geochemistry* 45, 191–197.

Tiyasha, Tung, T.M., Yaseen, Z.M., 2020. A survey on river water quality modelling using artificial intelligence models: 2000 – 2020. *J. Hydrol.* 585, 124670, 1–62.

Torab-mostaedi, M., Asadollahzadeh, M., Hemmati, A., Khosravi, A., 2015. Biosorption of lanthanum and cerium from aqueous solutions by grapefruit peel : equilibrium , kinetic and thermodynamic studies 559–573.

Yin, J., Zhang, X., 2020. Technologies for bHRPs and risk control, in: *High-Risk Pollutants in Wastewater*. Elsevier Inc., pp. 237–258.

Zhao, Q., Wang, Y., Xu, Z., Yu, Z., 2021. The potential use of straw-derived biochar as the adsorbent for La(III) and Nd(III) removal in aqueous solutions. *Environ. Sci. Pollut. Res.* 47024–47034.

Zheng, X., Liu, E., Zhang, F., Dai, J., Yan, Y., Li, C., 2017. Selective adsorption and separation of gadolinium with three-dimensionally interconnected macroporous imprinted chitosan films. *Cellulose* 24, 977–988.

Zhu, Y., Fan, W., Zhou, T., Li, X., 2019. Removal of chelated heavy metals from aqueous

solution : A review of current methods and mechanisms. *Sci. Total Environ.* 678, 253–266.