

Article

Uptake and Effects of Yttrium on the Seaweed *Ulva* sp.: A Study on the Potential Risks of Rare Earth Elements in Aquatic Environments

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Abstract

Technological proliferation relies on critical elements such as rare earth elements (REEs) and raises environmental problems associated with raw material extraction, industrial manufacturing, and the informal management of the growing e-waste. Algae are good bioindicators of contamination and have shown promise as biosorbents for remediating metal-contaminated environments. However, the effects of REEs on algae are still poorly documented and understood. This study investigated the uptake of yttrium (Y)—one of the most frequently used REEs, particularly in fluorescent lamps—by *Ulva* sp., as well as its effects on total chlorophyll content, relative growth rate, and biochemical performance. The algae were exposed to Y for 72 h at environmentally relevant concentrations, including levels found in e-waste leachates (0.5, 5.0, 50, and 500 mg/L). *Ulva* sp. removed 86% of Y within 72 h, with a bioconcentration factor of up to 621. EDTA analysis revealed that over 90% of yttrium was retained on the algal surface. SEM-EDS mapping showed crystal structures on the algae where Y was present. While the relative growth rate was unaffected by the tested Y gradient, photosynthesis was significantly impaired at 500 mg/L. Despite the activation of defence mechanisms, cell damage was observed at most Y concentrations tested, highlighting the potential risks associated with the presence of REEs in aquatic environments.

Keywords: rare earth elements; seaweed; bioconcentration; EDTA; oxidative stress



Academic Editor: Christos S. Akrotos

Received: 1 September 2025

Revised: 7 October 2025

Accepted: 14 October 2025

Published: 21 October 2025

Citation: Viana, T.; Colónia, J.;

Tavares, D.S.; Andrade, M.; Ferreira, N.; Freitas, R.; Pereira, E.; Henriques, B.

Uptake and Effects of Yttrium on the Seaweed *Ulva* sp.: A Study on the Potential Risks of Rare Earth Elements in Aquatic Environments. *Water* **2025**, *17*, 3023. <https://doi.org/10.3390/w17203023>

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1. Introduction

The ever-increasing use of rare earth elements (REEs) in high-tech products and new technologies has led to an increase in mineral exploitation [1], which has detrimental effects on the environment [2]. At the same time, the increase in waste electricals and electronic equipment (e-waste), which is often improperly disposed of due to scarce recycling protocols and high costs associated with its legal disposal [3], has also contributed to the leaching of these elements and other contaminants into the aquatic environment [4]. Studies have shown that the concentration of REEs in the Pearl River in China increased from 1 µg/L to 3007 µg/L, and that 33 major eastern rivers reached total suspended REE concentrations of approximately 1712 mg/L [5]. Water from the mining areas of Kerman in Iran (ΣREEs

concentration up to 10,646 µg/L) [6] and Wisniowka in Poland (Σ REEs concentration of 589 µg/L–90.1 mg/L) [7,8], or industrial discharges into the Rhine River in Germany (Σ REEs of 52 mg/L), are further examples of anthropogenic REE contamination [9]. Compared to 2013, the concentrations of some REEs increased by 37% in the Rhine and 50-fold in the Thames, London [10]. Among REEs, yttrium (Y) is one of the most widely used, particularly in LEDs and fluorescent lamps—where it is the REE present at the highest concentrations—as well as in catalysts, medical imaging devices, and other applications [11]. The presence of yttrium in aquatic environments has been shown to be detrimental, negatively affecting the growth rate of *D. magna* ([Y] > 790 µg/L) and *C. riparius* ([Y] > 450 mg/Kg), and increasing lethality of *D. magna* ([Y] > 1100 µg/L) and *O. mykiss* ([Y] > 450 µg/L) [12]. Microalgal growth was inhibited at Y concentrations greater than 0.5 mg/L, and the activity of the antioxidant enzyme superoxide dismutase increased with the addition of Y (0–0.30 mg/L). There is evidence that the toxicological effects of Y are related to its chemical similarity to Ca, acting as a potential Ca channel blocker, although most of the mechanisms underlying these effects remain unclear [13]. Moreover, the long-term effects of REEs in aquatic ecosystems are not yet fully understood or regulated, as there are currently no specific guidelines for REE concentrations in water [13]. The available studies are mainly limited to microalgae, bacteria, and invertebrates, and to our knowledge, no studies have investigated Y toxicity in marine macroalgae.

Marine macroalgae such as *Ulva* sp. are not only more complex than microalgae, but also act as biofilters in ecosystems by sorbing elements from the water column, and they form an important group of biomonitoring organisms [14]. Assessing Y accumulation, toxicity, and the underlying mechanisms in marine macroalgae could provide valuable information to policymakers for establishing safe levels of this element in aquatic ecosystems.

In this context, the present study aimed to evaluate (i) the biosorption and bioaccumulation of Y by the seaweed *Ulva* sp. at various concentrations, ranging from realistic environmental levels to those found in e-waste leachates; (ii) the effects of Y on *Ulva* sp. (as a bioindicator species) in terms of total chlorophyll content, relative growth rate, and biochemical performance; and (iii) the distribution of Y within the algae (bioadsorbed vs. bioaccumulated) and the possible mechanisms involved in the uptake process.

2. Materials and Methods

2.1. Reagents

All reagents used were of analytical grade. Nitric acid (65% (w/w), Suprapur[®]) was purchased from Merck (Darmstadt, Germany) and yttrium(III) chloride hexahydrate salt was purchased from Sigma-Aldrich (Darmstadt, Germany). All glassware was acid-washed before use.

2.2. Sampling

Ulva sp., an euryhaline seaweed [15], was harvested at low tide in the Aveiro estuary and transported to the laboratory in isothermal plastic bags containing local water. To remove surface sediments and potential epibionts, the seaweed was rinsed with seawater and placed in 20 L aerated aquaria under natural sunlight (positioned near a large window–12 h photoperiod) at room temperature (20 ± 2 °C).

Seawater was collected from the Atlantic Ocean near Praia da Barra in Aveiro, Portugal (40.644250, −8.745356), filtered through 0.45 µm Millipore filters and characterized (Supplementary Material-Table S1). Subsequently, a dilution with ultrapure water (Milli-Q, 18 MΩ/cm) to a salinity of 10 g/L was performed for seaweed acclimation and assays. Salinity at the sampling site varies between 35 and nearly 0 g/L [16], mainly due to tidal and river inputs; therefore, an intermediate salinity was chosen for this study. Salinity was

measured using an Eclipse model 45-63 portable refractometer (Bellingham + Stanley Ltd., Kent, UK).

2.3. Experimental Design

Uptake and toxicity of Y were assessed by exposing *Ulva* sp. (6 g/L fresh weight) to saline water (salinity 10 g/L; pH 7.8) contaminated with different initial concentrations of the element (0.5, 5.0, 50, and 500 mg/L) for three days. These acute exposure conditions were designed to simulate near-point-source pollution scenarios, such as landfill leachates or electronic waste disposal sites. Yttrium concentrations were chosen based on values reported in contaminated water systems [17], with the highest levels observed in leachates from fluorescent lamps [18,19]. While some tested concentrations exceed typical background levels in contaminated sites, including estuarine systems, this approach provides valuable insight into potential risks under worst-case exposure scenarios.

Assays were conducted in 1 L Schott Duran[®] flasks at room temperature (20 ± 2 °C) under natural sunlight (12 h light: 12 h dark). The spiked solutions were allowed to pre-equilibrate for 12 h before exposure. Sampling of the solution was performed immediately before the addition of the seaweed (0 h) and at predetermined exposure times. Samples were acidified with 65% HNO₃ to maintain a pH below 2 and stored at 4 °C until analysis.

All experimental conditions were performed in triplicate. Control solutions (mono-solutions of each Y concentration without seaweed) were run in parallel to assess possible Y losses. Additionally, blank solutions (seawater at salinity 10 g/L and seaweed without Y) were included to evaluate seaweed behaviour and baseline toxicity. pH monitoring was conducted *ex situ* after 6, 24, 48, and 72 h.

2.4. Analytical Quantification

Yttrium quantification in water samples was performed by inductively coupled plasma optical emission spectrometry (ICP-OES) using a Jobin Yvon Activa M (HORIBA Jobin Yvon, Edison, NJ, USA) spectrometer according to the methodology validated by Carvalho et al. [20]. The limit of quantification was defined as the lowest calibration standard (100 µg/L) and the detection limit was considered 1/3 of the quantification limit (33 µg/L). Calibration curves with a correlation coefficient below 0.999 were discarded. An acceptable coefficient of variation among replicates was set at 5%.

Yttrium quantification in seaweed samples was performed by ICP-OES following microwave-assisted acid digestion. Lyophilized seaweed samples (50–150 mg) were digested using a CEM MARS 6 (CEM Corporation, Matthews, NC, USA) microwave according to the protocol described by Viana et al. [21]. The digests were collected in polyethylene bottles to a final volume of 25 mL and diluted to volume with ultrapure water. Method quality control was ensured through the parallel analysis of procedural blanks (reaction vessels containing only reagents), which were consistently below the limit of quantification, and certified reference material (NIST 1515-Apple Leaves), with recoveries ranging from 88 to 109%.

2.5. Seaweed Characterization

The morphological analysis of *Ulva* sp. was made using a field emission gun (FEG) scanning electron microscope (SEM) (Model SU70; Hitachi, Tokyo, Japan), operated at 15 kV and equipped with an energy-dispersive X-ray spectroscopy (EDS) accessory (EDS Detector: Bruker AXS, Software: Quantax 400).

2.6. Partition of Y in Seaweed: Intra- vs. Extra-Cellular Concentration

Yttrium localization on *Ulva* sp. biomass (extracellular vs. intracellular) was evaluated using the desorbing agent ethylenediaminetetraacetic acid (EDTA). Based on the study by

Viana et al. [21], 0.001 mol/L EDTA solution in 0.6 mol/L NaCl was used as the extractant during 15 min at 150 rpm. The extracellular contaminant fraction (F_{ext} , %) was calculated considering the concentrations determined in the seaweed discs with ($q_{f\ EDTA}$, µg/g) and without (q_f , µg/g) prewash with EDTA (Equation (1)):

$$F_{ext}(\%) = \frac{q_f - q_{f\ EDTA}}{q_f} \times 100 \quad (1)$$

The internalized fraction (F_{int} , %) was calculated as follows (Equation (2)):

$$F_{int}(\%) = 100 - F_{ext} \quad (2)$$

2.7. Biological Responses

2.7.1. Biochemical Markers Analysis

Biochemical alterations due to Y exposure were identified by biochemical marker analysis related to antioxidant enzymes (superoxide dismutase, SOD; catalase, CAT; glutathione peroxidase, and GPx activities), biotransformation enzymes (glutathione S-transferase, GSTs activity) and cellular damage (lipid peroxidation, LPO levels) determined in the whole seaweed mass. These biomarkers were selected based on their well-established roles in algal ecotoxicology [22–24]: SOD, GPx and CAT are central to mitigating reactive oxygen species (ROS) accumulation, while GSTs contribute to detoxification and defence against metal-induced oxidative stress. LPO, in turn, serves as a marker of membrane lipid damage. Together, these biomarkers provide complementary insights into both the defence capacity of the organism and the extent of cellular damage following metal contamination.

For each biomarker determination, 0.25 g fresh weight (FW) seaweed was used, and the determination was performed in a total of 9 samples per treatment (three samples per replicate, and three aquaria per treatment). Technical duplicates of each sample were included in each analysis. All measurements were performed using a microplate reader (BioTek, Synergy HT). Samples extraction was performed in the appropriate buffer for each parameter at a ratio of 1:4 (m/v). The homogenization step was performed using a TissueLyzer II (Qiagen) set to a frequency of 20 1/s, for 180 s and centrifuged for 25 min at $10,000 \times g$ at 4 °C. Supernatants were stored at −80 °C or used immediately. For most measures, a potassium phosphate buffer—50 mmol/L at pH 7.0 with 1 mmol/L ethylenediaminetetraacetic acid, 1% (v/v) Triton X-100, and 1 mmol/L dithiothreitol—was used for sample extraction. For LPO, the extraction was performed using 20% (w/v) trichloroacetic acid (TCA).

Antioxidant and Biotransformation Enzymes

Superoxide dismutase (SOD) and catalase (CAT) activities were quantified using the methods described by Beauchamp and Fridovich [25] and Johansson and Borg [26], respectively. Standards were used for the quantification of SOD (0–60 U/mL SOD) and CAT (0–150 µM formaldehyde). SOD and CAT reactions were read at 560 nm and 540 nm, respectively. One unit (U) of enzymatic activity represents a 50% reduction in nitroblue tetrazolium (NBT) to SOD, and the formation of 1 nmol of formaldehyde per minute to CAT.

The determination of GSTs was performed according to Habig et al. [27] with modifications made by Carregosa et al. [28] and GPx quantification according to McFarland et al. [29] with modifications. The enzymatic reactions were read in a microplate reader at 340 nm for 5 min at 15 s intervals. The amount of thioether formed was calculated for the activity of GSTs using the extinction coefficient (ϵ) $9.6\ (\text{mM})^{-1}\ \text{cm}^{-1}$ and the conversion of nicotinamide adenine dinucleotide phosphate (NADPH) to GPx activity using the co-

efficient of extinction (ϵ) $6.22 \text{ (mM)}^{-1} \text{ cm}^{-1}$. The results were expressed as U per g FW, where U corresponds to the amount of enzyme that causes the formation of $1 \text{ }\mu\text{mol}$ of dinitrophenyl thioether per min for GSTs or the conversion of $1 \text{ }\mu\text{mol}$ NADPH per min in the case of GPx.

Oxidative Damage

Lipid peroxidation (LPO) levels were measured to assess oxidative damage to lipids, following the methods of Ohkawa et al. [30] modified by Carregosa et al. [28]. The final reactions were read at 535 nm, using the extinction coefficient for malondialdehyde (MDA) of $156 \text{ (mM)}^{-1} \text{ cm}^{-1}$. Results were expressed in nmol MDA equivalents per g of FW.

2.7.2. Total Chlorophyll Analysis

Total chlorophyll analysis was performed with a SPAD-502 m (Konica-Minolta, Japan). Because the leaf is not cut or otherwise damaged, the total chlorophyll of the same leaf can be quantified as the plant grows. Five independent SPAD measurements were performed per replicate disc (3 discs per flask).

2.7.3. Relative Growth Rate Analysis

Analysis of the relative growth rate (RGR, %/day)–Equation (3)–was performed after 24, 48 and 72 h of testing based on the area of digitalized discs (initial area $\bar{O} = 2.2 \pm 0.1 \text{ cm}$) calculated by the Fiji software 2.1.0.

$$GR(\%/day) = \frac{\ln A_t - \ln A_0}{t} \times 100 \quad (3)$$

where A_t refers to the area at time t (t in days) and A_0 refers to the initial area.

2.8. Data Analysis

The amount of Y removed from the solution by the seaweed (%) was calculated based on Equation (4):

$$\text{Removal}(\%) = 100 \times \frac{(C_0 - C_t)}{C_0} \quad (4)$$

where C_0 is the initial concentration of Y in solution (mg/L) and C_t is the concentration of Y at time t (mg/L). The average amount of element sorbed by the seaweed per unit mass, q_{real} (mg/g, dry weight (DW)), after 72 h, was measured by ICP-OES in the biomass after microwave-assisted acid digestion.

The bioconcentration factor (BCF, L/kg), defined as the ratio between the Y concentration in the seaweed after exposure (q_{real} , mg/kg) and the initial concentration of Y in water (C_0 , mg/L), was calculated as follows (Equation (5)):

$$BCF = \frac{q_{real}}{C_0} \quad (5)$$

The results of all biomarkers (SOD, CAT, GSTs, GPx and LPO) of each exposure condition were subjected to hypothesis testing using multivariate permutational analysis of variance using PERMANOVA complement in PRIMER v6 [31]. Pairwise comparisons between the conditions were made because significant differences were observed in the main test. Values were considered significantly different if the p -value was <0.05 (all p -values are reported in Table S2). Degrees of freedom = 5. The null hypothesis was that for each biomarker, there were no significant differences between the exposure concentrations. Significant differences between the conditions were represented in Figures with different lower-case letters.

Graphic illustrations were developed with GraphPad Prism 8.0.1.

3. Results

3.1. Yttrium Concentration in Water

For the conditions studied, the concentrations of Y measured in water immediately after contamination were: 0.41 ± 0.01 mg/L (0.5 mg/L nominal), 3.96 ± 0.06 mg/L (5.0 mg/L nominal), 40.2 ± 0.3 mg/L (50 mg/L nominal), and 463 ± 5 mg/L (500 mg/L nominal). A relative stability of C_t/C_0 ratios over 72 h was observed in the absence of *Ulva* sp. (control experiments), always within the control limits (Figure 1A) under all conditions. In contrast, the kinetic profiles of Y in the seaweed-based assays (Figure 1B) showed a rapid decrease in Y concentration in seawater during the first 9 h for the initial concentrations of 0.5 and 5.0 mg/L (resulting in removal efficiencies of 64% and 57%, respectively), followed by a slower decline, reaching near-equilibrium at 72 h with removal efficiencies exceeding 70%. For the higher exposure concentrations, removal after 72 h was 30% for 50 mg/L and between 3% and 5% for 500 mg/L.

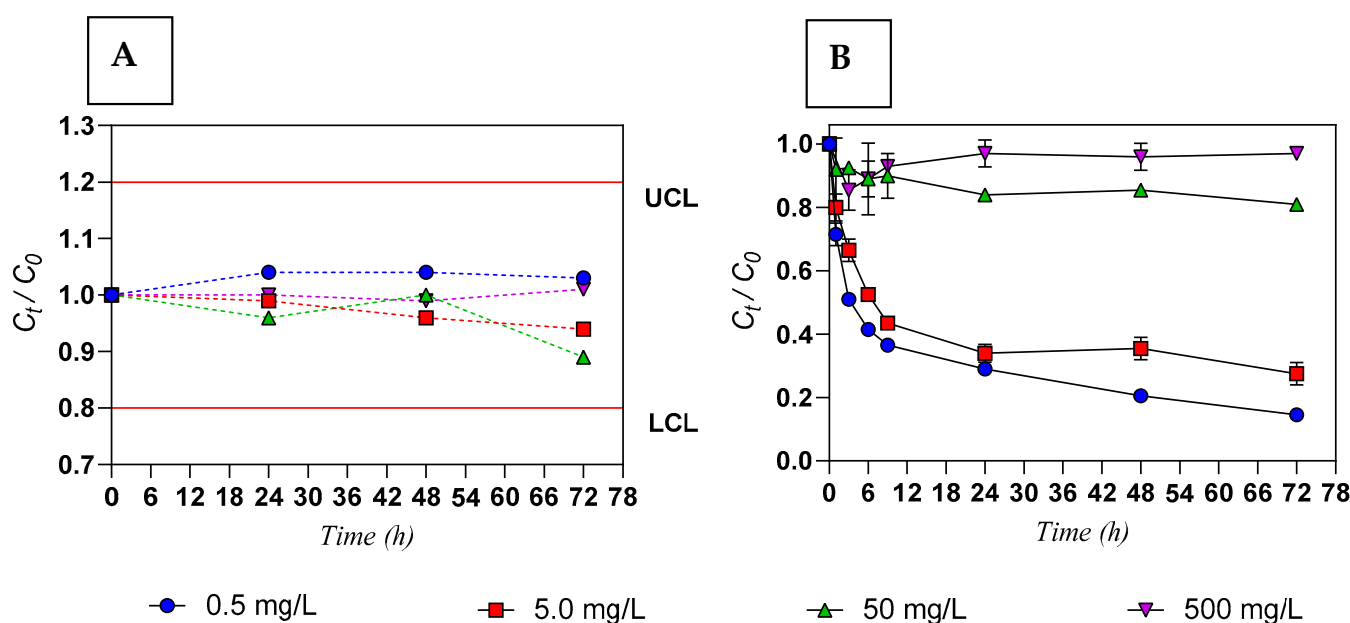


Figure 1. Ratio between the concentrations of Y at time t (C_t) and at initial condition (C_0) in contaminated water over 72 h: (A) in absence of seaweed (control—dashed lines); (B) in contact with living seaweed (*Ulva* sp.—solid line). Results are expressed as mean values and error bars represent the standard deviation ($n = 3$). Initial concentration of Y of 0.5; 5.0; 50; 500 mg/L; initial seaweed density of 6.0 g/L (FW); salinity 10 g/L. Upper control limit (UCL; $C_t/C_0 = 1.2$) and lower control limit (LCL; $C_t/C_0 = 0.8$) are depicted as a red line.

3.2. Yttrium Concentration in *Ulva* sp.

Table 1 presents the concentration of Y in *Ulva* sp. biomass determined by ICP-OES after 72 h of exposure to spiked water. The baseline concentration of Y, measured in pristine seaweed immediately after field collection, was below 0.02 mg/g, which was also observed in seaweed from the blank assays (seaweed exposed to clean seawater) after 72 h. The bioconcentration factor (BCF) varied with the initial Y concentration in water, ranging from 621 to 42 L/kg, with lower BCF values recorded at higher exposure concentrations.

Table 1. Concentration of Y in the biomass of *Ulva* sp. at the end of the exposure (q_{real} , mg/g), and the respective bioconcentration factor (L/kg), for the different initial concentrations of Y studied.

[Y] (mg/L)	<i>Ulva</i> sp.				
	Blank	0.5	5.0	50	500
q_{real} (mg/g)	<0.02	0.237 ± 0.098	3.11 ± 0.17	8.12 ± 0.35	20.9 ± 1.3
BCF (L/kg)	-	474 ± 197	621 ± 33	162 ± 7.0	42 ± 2.7

3.3. Yttrium Partition in *Ulva* sp. Biomass

To distinguish between surface sorption and intracellular uptake, the seaweed was subjected to EDTA extraction, which revealed that 92–96% of the Y was bound to the surface of *Ulva* sp. (Figure 2).

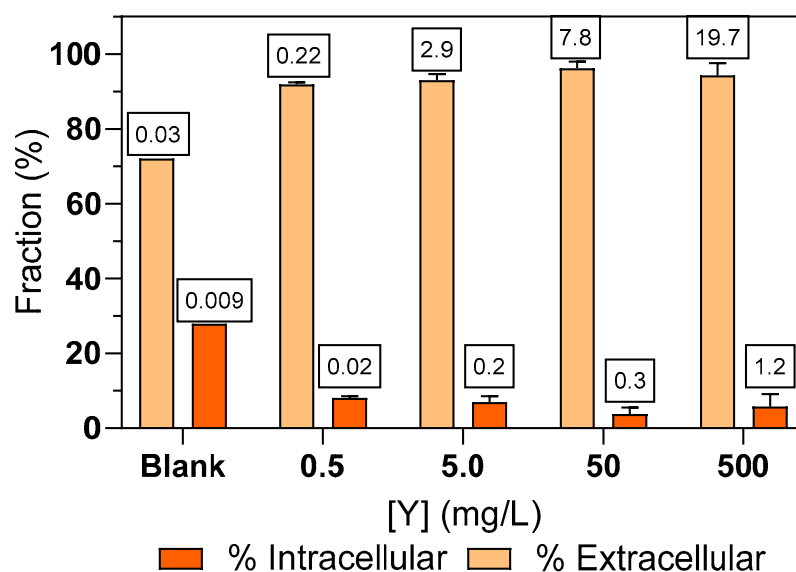
**Figure 2.** Extracellular and intracellular fractions of Y in *Ulva* sp. after 72 h exposure to different initial Y concentrations in water (blank; 0.5; 5.0; 50; 500 mg/L), operationally defined by extraction with 0.001 mol/L EDTA L in 0.6 mol/L NaCl. Values inside the square indicate the amount of Y ($\mu\text{g/g}$) inside and outside *Ulva* sp.

Figure 3 presents scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM-EDS) images of *Ulva* sp. before (A and B) and after exposure (C and D) to Y at 500 mg/L. Changes were observed on the seaweed surface after exposure. SEM-EDS analyses revealed heterogeneous surface deposits enriched in Y. These deposits displayed well-defined, angular morphologies that resemble crystal-like precipitates (Figure 3C,D).

3.4. Biological Responses

3.4.1. Antioxidant and Biotransformation Enzymes

The activity of SOD decreased significantly in the seaweed exposed to Y concentrations of 0.5; and 500 mg/L compared to seaweed from the blank condition. In contrast, the SOD activity in seaweed exposed to 5.0 and 50 mg/L Y was significantly higher than in the blank and the highest concentration (Figure 4A).

Seaweeds exposed to all concentrations of Y showed significantly lower CAT activities compared to seaweed Y-free (blank condition, Figure 4B).

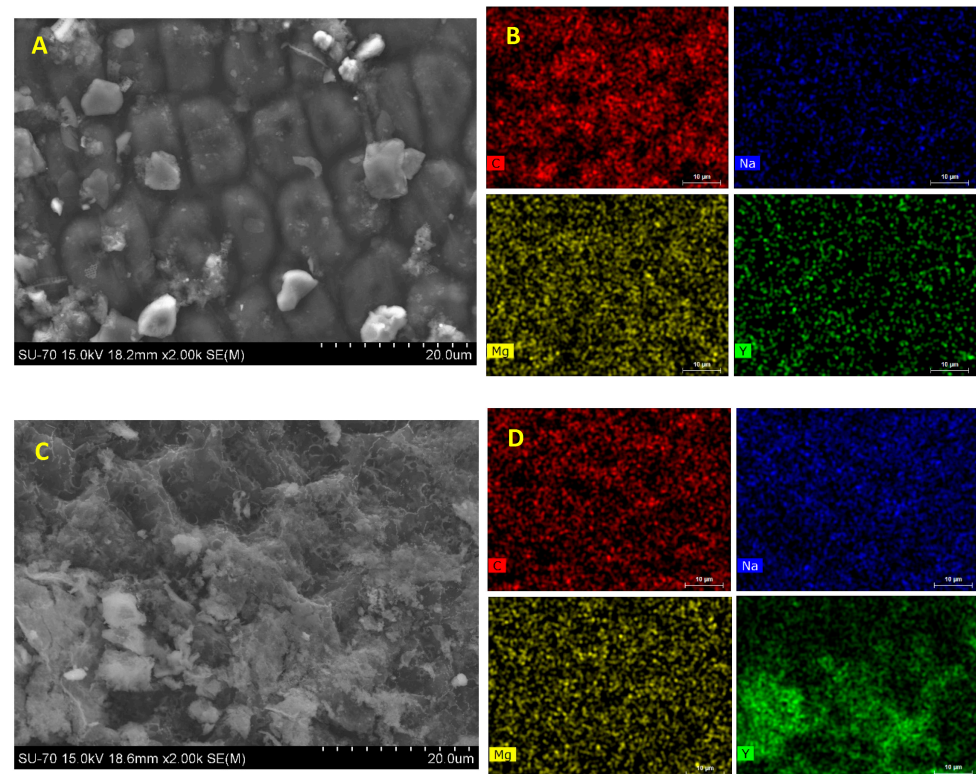


Figure 3. Scanning electron microscopy images (A,C) and respective energy dispersive X-ray mapping of main elements (B,D) on *Ulva* sp. surface before (A,B) and after (C,D) exposure to 500 mg/L of Y (Carbon–red, Sodium–blue, Magnesium–yellow, Yttrium–green).

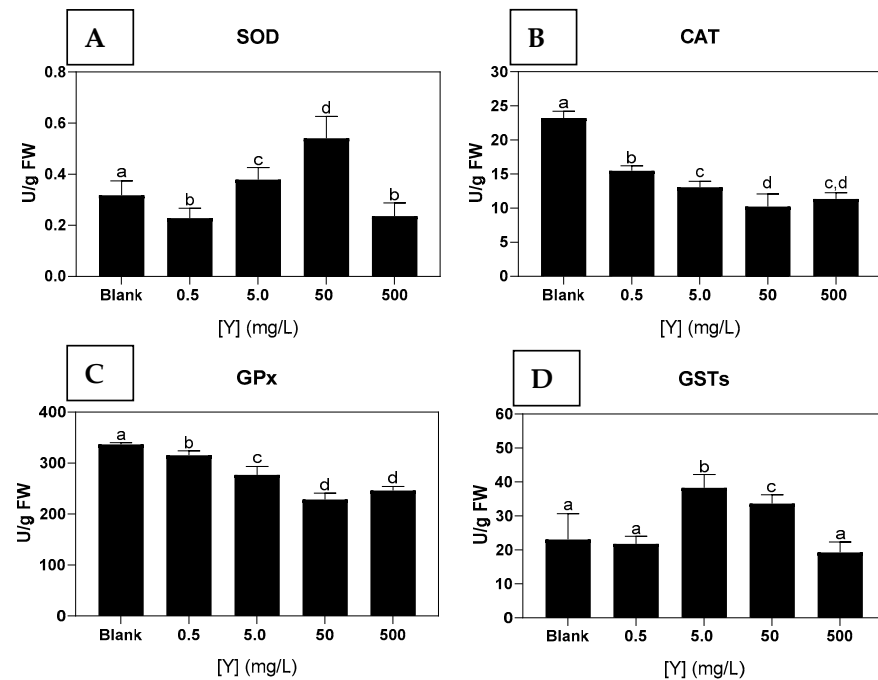


Figure 4. (A)–Superoxide dismutase (SOD); (B)–Catalase (CAT); (C)–Glutathione peroxidase (GPx); (D)–Glutathione-S-transferases (GSTs) activities in *Ulva* sp. from the blank condition and after exposure to different concentrations of Y (0.5; 5.0; 50; 500 mg/L) for 72 h. Results are expressed as mean and standard deviation ($n = 9$). Different letters indicate significant differences ($p < 0.05$) between the conditions.

The GPx activity was significantly lower in seaweeds exposed to all Y concentrations compared to unexposed ones. The lowest GPX activity was observed in seaweed exposed to 50 and 500 mg/L Y (Figure 4C).

Seaweeds exposed to Y concentrations of 5.0 and 50 mg/L showed a significant increase in GSTs's activity compared to the blank values. No significant differences were observed among seaweeds exposed to 0.5, 500 mg/L and the blank condition (Figure 4D).

3.4.2. Oxidative Damage

Lipid peroxidation (LPO) levels increased significantly in seaweed exposed to all Y concentrations compared to the uncontaminated control (blank condition), with the highest levels observed in seaweed exposed to Y concentrations of 5.0, 50, and 500 mg/L (Figure 5).

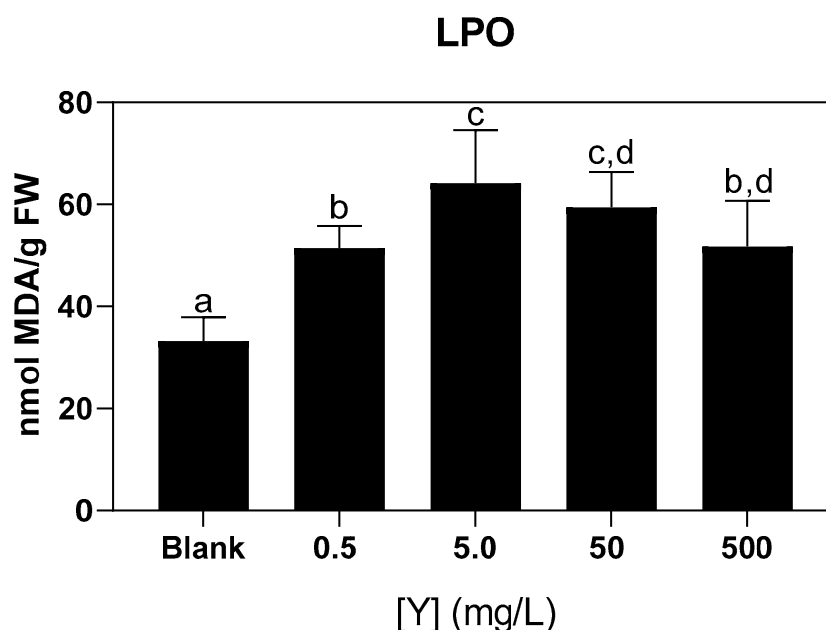


Figure 5. Lipid peroxidation (LPO) levels in *Ulva* sp. from the blank condition and after exposure to different concentrations of Y (0.5; 5.0; 50; 500 mg/L) for 72 h. Results are expressed as mean and standard deviation ($n = 9$). Different letters indicate significant differences ($p < 0.05$) between the conditions.

3.4.3. Total Chlorophyll, Relative Growth Rate and pH Monitoring

After 3 days of exposure to different Y concentrations, total chlorophyll content decreased significantly at intermediate and high concentrations (5.0–500 mg/L) compared to the blank, although no visible chlorosis was observed (Figure S1A), with SPAD values of 5.89 ± 0.07 , 5.49 ± 0.84 , and 5.74 ± 0.45 for 5.0, 50, and 500 mg/L, respectively. In contrast, no significant differences were found between the lowest exposure concentration (0.5 mg/L Y, 9.53 ± 0.05 SPAD) and the blank condition (9.69 ± 0.05 SPAD).

The relative growth rate (RGR, %/day) of seaweed exposed to Y concentrations decreased transiently after 24 and 48 h at the higher concentrations (5.0 and 500 mg/L) by approximately 1.6-fold and 2.6-fold, respectively, compared to the Y-free condition (blank condition) (Figure S1B). However, at 72 h, the RGR for all Y concentrations was not significantly different from the blank condition.

Monitoring of medium pH revealed concentration-dependent differences consistent with photosynthetic activity (Figure 6). While pH remained stable at the blank (clean seawater with *Ulva* sp.) and at 0.5 mg/L Y, it declined notably at 500 mg/L Y.

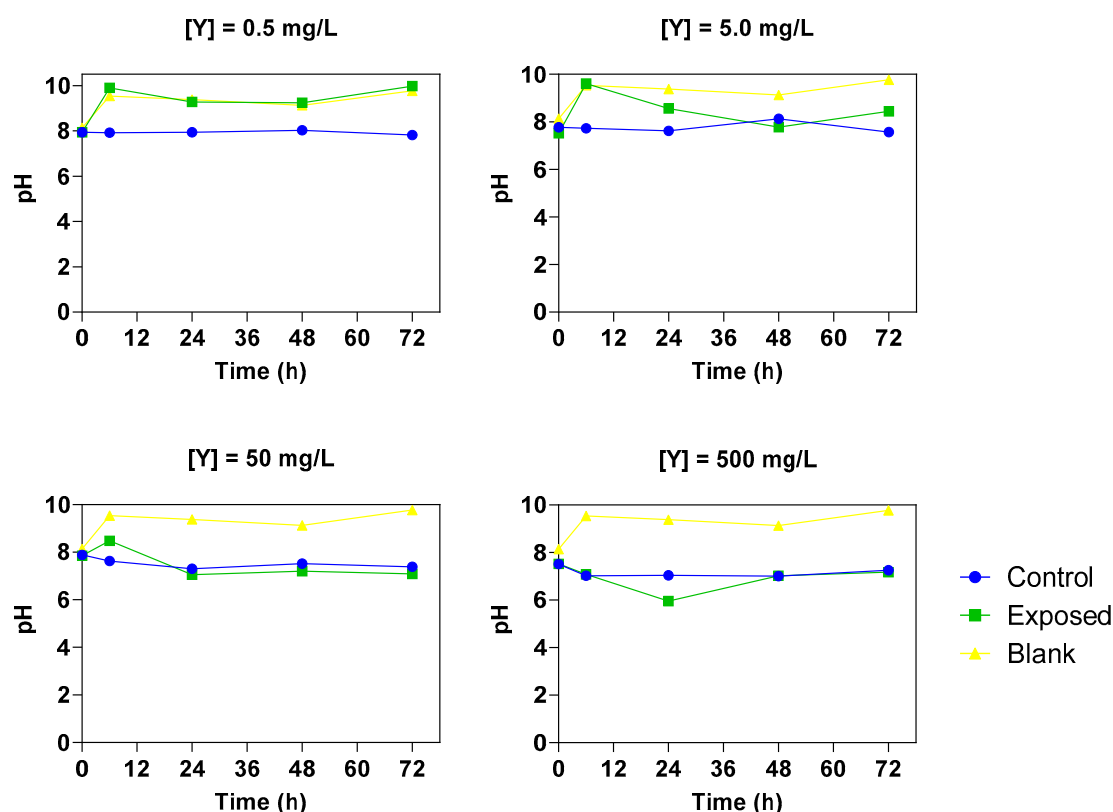


Figure 6. pH monitoring of different concentrations of Y (0.5; 5.0; 50; 500 mg/L) over 72 h of (1) seaweed *Ulva* sp. exposed to Y (contaminated seawater-green); (2) control solutions (spiked seawater without *Ulva* sp.-blue); and (3) blank solutions (clean seawater with *Ulva* sp.-yellow). Amount of seaweed of 6.0 g/L (FW); salinity 10 g/L; initial pH 7.8. Results are expressed as mean and standard deviation ($n = 3$).

4. Discussion

In contrast to other metals, available information on the toxicity of Y is extremely limited, particularly in seaweed. This knowledge gap underscores the need for fundamental baseline studies to elucidate its specific impacts at both physiological and ecological levels. Addressing this gap is therefore essential for improving predictive models of metal mixture toxicity and for guiding future strategies in environmental monitoring and management.

4.1. Sorption of Y by *Ulva* sp.

A rapid decrease in Y concentrations in water was observed during the first 9 h of contact with *Ulva* sp., likely due to the steep initial concentration gradient, as the seaweed surface was essentially Y-free (baseline levels < 0.02 mg/g, consistent with Pinto et al. [32]). Generally, sorption is a passive process where cations diffuse to available binding sites and are adsorbed. Protein- and polysaccharide-derived hydroxyl, amine, and carboxyl groups may be involved in the sorption of Y by *Ulva* sp. [33]. In metabolically active sorbents such as living seaweed, this is followed by metabolism-dependent internal accumulation until a steady state is reached, a well-known nutrient uptake mechanism [34,35]. Although higher initial concentrations enhance interactions with active sites, at 500 mg/L the binding sites became saturated, leading to lower removal efficiencies (3–5%). However, the highest concentration of Y in seaweed biomass was recorded under this condition, reaching 20.9 ± 1.3 mg Y/g. The strong capacity of *Ulva* sp. to uptake Y has been previously reported by Pinto et al. [36] at exposure concentrations of 0.5 mg Y/L. In this study, BCFs ranged from 42 to 621 L/kg, showing an inverse relationship with the water concentration

gradient, highlighting that metal accumulation in seaweed tissues responds directly to the surrounding Y levels [37].

4.2. Fractioning of Y in *Ulva* sp. Biomass

Extractions with the chelating agent EDTA revealed that over 90% of the Y was located in the extracellular fraction of the seaweed. This high proportion may be influenced by the short duration of the experiments relative to the slower rate of Y internal accumulation in the seaweed. The interactions between REEs and living organisms remain understudied; however, a study on *Nymphoides peltata* (a freshwater plant) showed that Y was predominantly located in the cell wall (73–86%), with smaller amounts in organelles (8–19%) and the soluble fraction (4–8%) [38].

Elemental mapping (SEM-EDS) revealed distinct Y-enriched deposits on the algal surface at high exposure concentrations, supporting the conclusion that Y was predominantly located in the extracellular fraction. Although these deposits were initially described as ‘crystal-like,’ this refers to their morphology under SEM rather than crystallographic confirmation. Their heterogeneous, particulate appearance is consistent with surface precipitation or aggregation phenomena that occur once binding sites are saturated [21]. Similar surface-associated patterns have been reported in other algal systems. For example, Tunali & Yenigun [39] observed that Nd^{3+} accumulated primarily on the surface of living *C. vulgaris* with a scattered distribution revealed by SEM, although a portion also entered the intracellular space. This reinforces the notion that REEs often accumulate preferentially at the cell surface, with limited but detectable penetration into internal compartments. The formation of such deposits may further contribute to oxidative stress by physically altering the cell wall surface, limiting ion exchange, and reducing photosynthetic efficiency.

The sorption mechanism of *Ulva* sp. may be analogous to that described by Liang and Shen [11] for *S. marcescens* exposed to Y, where Y ions are initially attracted to the surface through electrostatic interactions. In bacteria, cell wall peptidoglycan, lipopolysaccharides, and exopolymers provide negatively charged groups (carboxyl, hydroxyl, phosphate) that bind metals through ion exchange and complexation [40,41]. A similar situation occurs in *Ulva* sp. cell walls, where ulvan and other polysaccharides contain abundant sulphate and carboxyl groups that act as cation-binding sites, as confirmed by FTIR and SEM-EDS analyses in recent studies [21]. Although the molecular scaffolds differ between microbes and seaweed, the chemical basis—adsorption to anionic functional groups followed by potential internalization—is analogous. This makes microbial studies a relevant comparative model for understanding metal–alga surface interactions. Subsequently, Y ions near the cell surface may exchange with Na^+ , K^+ , Mg^{2+} , and other light metal ions through ion exchange and/or react with functional groups such as hydroxyl, carboxyl, and amine groups, forming stable complexes. A speciation analysis was performed using VisualMinteq software (version 3.1—Figure S2) to infer about the predominant chemical Y species in the spiked seawater. Under the experimental conditions studied, Y speciation revealed to be dominated mainly by carbonate species (YCO_3^+ and $\text{Y}(\text{CO}_3)_2^-$) across all tested concentrations, with an increased sulphate complexation at the highest concentration. These speciation patterns are consistent with the high affinity of REEs for carbonate in aquatic systems [42,43]. From a biological perspective, the predominance of carbonate complexes reinforces that binding to algal surfaces may occur mainly through interaction with negatively charged functional groups such as carboxyl, hydroxyl, and phosphoryl groups, which are abundant in algal cell walls [42]. The relatively low proportion of Y^{3+} in solution in concentrations 0.5–50 mg/L (4–7%) versus 500 mg/L (25%), could represent one of the main reasons explaining the higher toxicity at 500 mg Y/L, given that the trivalent forms should be the most bioavailable [43].

Our results show that, after the accumulation on the surface occurs, around 5–8% of the total Y is absorbed inside the cells, regardless of the initial exposure concentration. When expressed as mass concentration, this intracellular fraction represents a measurable but relatively small portion compared with the extracellular load. The proportion remained <10% across all tested concentrations, suggesting that intracellular accumulation may be limited by the transport mechanism rather than external availability. Similar patterns have been reported in recent works as those by Ordóñez et al. [41] which highlights in their review that living algal biomass often shows surface-binding (biosorption) as the dominant uptake mode under metal exposure, with intracellular uptake being metabolism-dependent and generally smaller in magnitude. Even in algae like *Ulva* exposed to non-REE metals (Cu, Zn, Co), fast surface adsorption dominates in early exposure phases, with limited additional mass gain intracellularly over time [44].

This indicates that while surface complexation is rapid and dominant, intracellular Y incorporation may be constrained by physiological barriers. This intracellular fraction is likely responsible for most of the biochemical alterations observed in the biomarker analyses. Once inside the cells, REEs can even alter organelle structures [12,45].

4.3. Biomarkers and Physiological Responses of *Ulva* sp. to Y Exposure

Organisms exposed to stressful conditions can experience increased production of reactive oxygen species (ROS), triggering a cascade of responses, including antioxidant strategies, to prevent cellular damage [46]. SOD activity is suppressed at the lowest and highest tested concentrations and elevated at the intermediate concentration. This pattern can arise when moderate stress triggers an adaptive up-regulation of defence enzymes (transcriptional activation/induction) [47], whereas both very low exposures (which may directly interfere with basal enzyme function or regulatory signalling) and very high exposures (which cause enzyme inactivation, cofactor depletion, or cell damage) result in reduced measured activity. Although SOD activity was stimulated at intermediate Y concentrations (5.0 and 50 mg/L), CAT and GPx activities were inhibited. This apparent contradiction can be reconciled because SOD converts superoxide to hydrogen peroxide (H_2O_2); an increase in SOD therefore raises the cellular H_2O_2 burden, which must be removed by CAT and GPx [48]. However, under Y exposure, these H_2O_2 -removing enzymes may be non-functional or depleted—for example via direct inhibition or disruption of enzyme active sites by REEs, depletion of reduced glutathione required by GPx, NADPH/cofactor loss that impairs catalase protection, or oxidative inactivation of the enzymes themselves—leading to an accumulation of H_2O_2 and the observed increase in lipid peroxidation [49]. Thus, rather than SOD suppression causing lower CAT/GPx, the more likely sequence is: (1) SOD up-regulation (attempt to remove $\text{O}_2^{\bullet-}$); (2) increased H_2O_2 formation; (3) insufficient CAT/GPx activity (due to inhibition/cofactor depletion/protein damage); (4) H_2O_2 /LPO accumulation. Nevertheless, the phase II biotransformation enzymes, GSTs, were activated at intermediate concentrations of Y. GSTs are a family of enzymes that play a crucial role in detoxifying both endogenous compounds, such as ROS, and exogenous compounds, including environmental toxins. Their activity depends on the conjugation of reduced glutathione (GSH) to electrophilic substrates, thereby increasing solubility and facilitating excretion. In algae, GSTs also act as an auxiliary antioxidant system by neutralizing lipid peroxidation products (e.g., 4-hydroxynonenal, malondialdehyde), which may accumulate when primary antioxidant enzymes such as CAT and GPx are inhibited.

The selective induction of GSTs at intermediate Y concentrations supports the idea that phase II biotransformation acts as an early adaptive response to moderate oxidative/chemical stress [47,50]. However, the lack of GST stimulation at the highest con-

centration likely reflects the limits of this defence—for example, depletion of reduced glutathione (GSH) or direct enzyme inhibition under severe stress—a pattern recently reviewed for microalgae and seen across aquatic organisms [47,51]. Moreover, *Ulva*-specific multi-endpoint studies show that antioxidant and physiological responses are highly dose- and tissue-dependent, supporting our interpretation that GST up-regulation can mitigate oxidative damage at moderate exposures but may be insufficient at very high contaminant loads [52].

This study demonstrates increased LPO levels at all tested concentrations, consistent with the EDTA and SEM analyses. Since most Y is located on the surface of the seaweed biomass, activation of endogenous defences is limited, making cellular damage more likely. Taken together, these results suggest a mechanistic cascade linking antioxidant enzyme inhibition, oxidative stress, and photosynthetic impairment. At intermediate Y concentrations, the activation of SOD reflects an early attempt to counteract ROS, whereas the inhibition of SOD, CAT, and GPx at other concentrations indicates a reduced overall antioxidant capacity. The consequent accumulation of ROS is consistent with the significant rise in lipid peroxidation, which signals oxidative injury to membranes, particularly in chloroplasts, where thylakoid integrity is essential for light harvesting and electron transport. Such oxidative damage provides a mechanistic explanation for the reductions in chlorophyll content and the altered pH patterns observed in the medium. Because pH dynamics are directly linked to CO₂ uptake during photosynthesis, the decline in pH at high Y concentrations reflects impaired photosynthetic activity caused by ROS-driven damage. In this way, biochemical and physiological endpoints can be understood as interconnected responses: antioxidant disruption leads to ROS accumulation, ROS causes lipid peroxidation, and this in turn compromises photosynthetic performance.

Figueiredo et al. [53] evaluated the toxicity of Lanthanum (La, 15 µg/L) and Gadolinium (Gd, 10 µg/L) to *Ulva rigida* during 7 days and reported that both REEs triggered an adequate activation of the antioxidant defence system, thereby avoiding lipid damage. The differing results may be due to the much lower concentrations tested by these authors—33 to 500 times lower than those used in the present study. Henriques et al. [54] evaluated the toxicity of Europium (Eu, 152 and 500 µg/L) to *Ulva lactuca* and the results showed toxicity but no cellular damage, likely due to the activation of the seaweed's defence mechanisms, combined with exposure to concentrations 3 to 33 times lower than those used in the present study. These results highlight the importance of exposure concentration on organism effects and warn of the risks associated with the increasing generation of electronic waste and related pollution, particularly concerning REEs.

Although chlorosis was not observed, total chlorophyll content decreased at intermediate Y concentrations (5.0–500 mg/L). This suggests that the energy cost of chlorophyll synthesis was impaired, potentially due to REEs interference with the chlorophyll active site, which can limit photosynthetic energy conversion.

Consistent with this mechanistic framework, the production of light-driven ROS can result in oxidative harm to crucial photosynthetic components, consequently blocking photosynthetic activity. This inhibition could manifest as a decrease in CO₂ assimilation, accompanied by an increase in leaf H₂O₂ production [55]. While some studies indicate that low concentrations of REEs may enhance photosynthetic activity [56,57], higher concentrations of REEs might compete with or replace Mg²⁺ due to their structural similarity, thereby potentially limiting or inhibiting photosystem activity [45,58,59]. In this study, the pH monitoring results are consistent with this interpretation, as reduced CO₂ uptake under high Y exposure coincided with signs of oxidative stress and chlorophyll loss, reinforcing the conclusion that photosynthesis was impaired under these conditions. This is because pH in the culture medium is closely tied to photosynthetic activity: as seaweed assimilate

dissolved inorganic carbon during photosynthesis, CO₂ removal and bicarbonate uptake typically lead to an increase in pH. Conversely, when photosynthetic performance is impaired, CO₂ drawdown is reduced and the medium tends to remain more acidic or even decrease in pH [60,61]. Thus, the observed pH decline at 500 mg/L Y strongly supports the conclusion that photosynthetic processes were disrupted under these conditions.

In summary, Y exposure in *Ulva* sp. triggered a cascade of effects in which antioxidant enzyme inhibition led to ROS accumulation, ROS drove lipid peroxidation, and this oxidative damage impaired photosynthetic function, providing a coherent mechanistic link from biochemical stress to physiological impairment.

5. Conclusions

This study evaluated the ability of living seaweed *Ulva* sp. to uptake yttrium (Y) from water under ecologically relevant contamination scenarios, including concentrations typical of e-waste leachates. Dose-dependent accumulation resulted in removal efficiencies up to 86% and bioconcentration factors (BCF) as high as 621 L/Kg. EDTA extraction showed that 92–96% of Y was located on the seaweed surface across most tested concentrations.

The relative growth rate (RGR) of *Ulva* sp. after 72 h of exposure was not negatively affected; however, at 500 mg/L, a decrease in pH was observed as well as signs of chlorosis, suggesting a significant impairment in photosynthesis. Biochemical marker analysis revealed oxidative stress and activation of antioxidant defences at intermediate Y concentrations, though these were insufficient to prevent cellular damage. Given that most Y resides on the seaweed surface (confirmed by EDTA and SEM analysis), endogenous defence activation is limited, increasing susceptibility to cellular harm.

These findings highlight the environmental risks posed by Y contamination for seaweed in aquatic and coastal ecosystems. Nevertheless, some limitations must be acknowledged: the short exposure duration (72 h), the use of a single seaweed species, and the controlled laboratory conditions may not fully capture the complexity of natural environments. Future research should therefore include longer-term exposure scenarios, a broader range of ecologically relevant seaweed, and experiments in more realistic mesocosms or field conditions. In addition, evaluating potential trophic transfer and chronic ecological effects would provide a more comprehensive understanding of the risks posed by Y and other REEs. By addressing these aspects, future studies can better inform environmental risk assessments and management strategies for REEs, particularly in regions affected by e-waste and industrial discharges.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w17203023/s1>, Figure S1: A—Total chlorophyll content (spad value); B—Relative growth rate (%/day) in the seaweed *Ulva* sp. exposed to different concentrations of Y (0.5; 5.0; 50; 500 mg/L). The results are the means and standard deviation (n = 9); Figure S2: Fraction (%) of aqueous speciation calculation using the VisualMinteq software for average composition of Y at different initial concentrations on real seawater (0.5; 5.0; 50; 500 mg/L). Salinity 10; initial pH 7.8; Table S1: Seawater parameters and major and minor elements concentration; Table S2: p-values obtained through pair-wise comparison tests performed on physiological and biochemical results using PERMANOVA routine from the software PRIMER (PERMANOVA + add-on in PRIMER v6).

Author Contributions: Methodology, R.F., E.P. and B.H.; Validation, T.V., J.C., D.S.T., M.A., N.F., R.F., E.P. and B.H.; Formal analysis, T.V., J.C., D.S.T., M.A., N.F., R.F. and B.H.; Investigation, T.V., J.C., D.S.T., M.A., N.F. and B.H.; Resources, E.P. and B.H.; Writing—original draft, T.V., R.F. and B.H.; Supervision, R.F., E.P. and B.H.; Project administration, E.P. and B.H.; Funding acquisition, E.P. and B.H. All authors have read and agreed to the published version of the manuscript.

Funding: This work received financial support from the European Union (FEDER funds) through the Operational Competitiveness Program (COMPETE2020)-project N°46998_N9ve-REE and from PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior). Thanks are due for the financial support to REQUIMTE (UID/50006-Laboratório Associado para a Química Verde-Tecnologias e Processos Limpos) and UID Centro de Estudos do Ambiente e Mar (CESAM) (+LA/P/0094/2020).

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Acknowledgments: Thainara Viana, and Nicole Ferreira thanks FCT/MCTES (Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) and ESF (European Social Fund) through NORTE 2020 (Programa Operacional Região Norte) for their PhD grant ref. 2022.13015.BD (<https://doi.org/10.54499/2022.13015.BD>), and 2022.13017.BD (<https://doi.org/10.54499/2022.13017.BD>), respectively.

Conflicts of Interest: The authors declare no conflict of interest.

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