applied sciences

MDPI

Article

Towards the Sustainable Removal of Heavy Metals from Wastewater Using *Arthrospira platensis*: A Laboratory-Scale Approach in the Context of a Green Circular Economy

Lamprini Malletzidou [1,2], Eleni Kyratzopoulou [1], Nikoletta Kyzaki [1], Evangelos Nerantzis [1] and Nikolaos A. Kazakis [1,*]

[1] Laboratory of Archaeometry and Physicochemical Measurements, Athena—Research and Innovation Center in Information, Communication and Knowledge Technologies, Kimmeria University Campus, P.O. Box 159, GR-67100 Xanthi, Greece; l.malletzidou@athenarc.gr (L.M.); e.kiratzopoulou@athenarc.gr (E.K.); nikoletta.kyzaki@athenarc.gr (N.K.); e.nerantzis@athenarc.gr (E.N.)

[2] Laboratory of Advanced Materials and Devices, School of Physics, Faculty of Sciences, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece

* Correspondence: nikkazak@athenarc.gr

Featured Application: Efficiency of microalga for the bioremediation of heavy metal-contaminated systems.

Abstract: The use of living *Arthrospira platensis* (*A. platensis*) cultures emerges as a promising green solution for the bioremediation of water contaminated by toxic metal waste. The scope of the present study is to evaluate the microalga's potential in heavy metal remediation, in the case of multi-metal-treated (multi-MT) systems. For this reason, *A. platensis* cultures were exposed to mono- and multi-metal solutions of Cu, Cd, Ni, Pb, and Zn, and their metal adsorption ability was investigated. The heavy metal removal efficiency of *A. platensis* cultures was evaluated using atomic absorption spectroscopy (AAS). Additionally, the cultures were examined using Fourier transform infrared (FTIR) spectroscopy, Near-Infrared (NIR) Spectroscopy, UV-Vis spectrophotometry, and optical microscopy, together with pH and electrical conductivity (EC) measurements to evaluate the quality of the cultures and the changes induced by heavy metal stress. The results showed that metal removal is still efficient in multi-MT cultures. In particular, Cu, Cd, Pb, and Zn removal of multi-MT cultures is elevated or relative to the respective removal of the mono-metal-treated (mono-MT) cultures, showing a synergistic or cooperative interaction between the metals, while the removal of Ni of multi-MT cultures decreased compared to Ni of mono-MT cultures, showing an antagonistic interaction to the other metals. The study shows that *A. platensis* is considered an effective microalga toward the bioremediation of multi-metal polluted cultures.

Keywords: microalgae; cyanobacteria; bioremediation; multi-metal waste; AAS; NIR; FTIR; FPA; chlorophyll-a; *Spirulina platensis*

check for updates

Academic Editor: Francesco Caridi

Received: 13 December 2024
Revised: 8 January 2025
Accepted: 13 January 2025
Published: 15 January 2025

Citation: Malletzidou, L.; Kyratzopoulou, E.; Kyzaki, N.; Nerantzis, E.; Kazakis, N.A. Towards the Sustainable Removal of Heavy Metals from Wastewater Using *Arthrospira platensis*: A Laboratory-Scale Approach in the Context of a Green Circular Economy. *Appl. Sci.* **2025**, *15*, 791. https://doi.org/10.3390/app15020791

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Water pollution by heavy metals has become a serious environmental and public health problem due to the induced toxicity, persistence, and bioaccumulation [1–3]. Even though Pb, Hg, Cd, and Cr are naturally released into the environment through processes such as the weathering of rocks, volcanic action, and erosion of sedimentary deposits, human activities have artificially increased their levels in severe proportions that seriously

threaten ecosystems and human health [4]. Heavy metal pollution mainly stems from industrial activities such as mining, smelting, and the manufacturing of electronics, while the use of fertilizers and pesticides in agricultural activities results in the leaching of metals into water systems [5]. Other contributors include tanneries, textiles, urban runoff, and coal combustion, adding to environmental contamination and enhancing the problem. These metals are non-biodegradable in nature and become bioaccumulated by aquatic organisms, where they are magnified through the food chain, posing serious health threats that include neurological damage, kidney failure, and cancer [6].

The conventional physicochemical techniques for heavy metal remediation include chemical precipitation, ion exchange, and adsorption. However, these techniques tend to be very costly, less effective, and cannot be viable for a long period [6]. At the same time, conventional techniques also create environmental problems, which include either the generation of secondary contaminants or the use of huge amounts of energy [7]. Adsorption—whereby metals are attached to the surface of materials such as activated carbon and biochar—is a cost-effective approach for small-scale applications. Chemical precipitation involves the transformation of dissolved metal ions into insoluble forms, which can then be more easily extracted; however, this process tends to produce large quantities of solid waste [8]. The physicochemical methods of coagulation and flocculation favor the aggregation of small size metal particles into big clusters, which could then easily be separated, but generally require chemical additives, which potentially modify the water chemistry itself [9]. Advanced membrane technologies, like reverse osmosis and electrodialysis, are very accurate but involve the production of concentrated brine wastes, creating disposal problems [10]. The appropriate remediation method is selected based on the cost, scalability, and environmental impacts, which calls for site-specific solutions and the integration of hybrid technologies to achieve optimal results [11].

Therefore, a dire need has emerged for the adoption of novel and cost-effective techniques capable of mitigating the heavy metal burden in an efficient manner. To this direction, various green remediation techniques were proposed for the removal of heavy metals by algae. Microalgae have emerged as efficient tools in heavy metal remediation because of their unique characteristics of absorbing and accumulating metals from contaminated water, being an environmentally friendly and economically viable option at the same time [1–3,6]. Microalgae have the potential for a very innovative and green approach to the removal of heavy metals like Pb, Hg, Cd, Zn, and Ni from contaminated water [1,3,4,6,12–22]. Moreover, concerning circular economy, microalgae bioremediation helps not only to clean the environment, but it also provides value-added byproducts such as biofuels and other bio-based materials [23]. The application of microalga-based remediation to wastewater treatment systems was highlighted in several works as a promising tool [23]. In light of such advancements, it is important to explore the microalgae potential for the elaboration of environmentally enhancing processes for heavy metal control, wastewater treatment, and for sustainable biofuel production. Innovative approaches of using microalgae could pave ways toward more sustainable and efficient paths to reduce the harmful effect of heavy metal pollution and enable a clean and healthy environment [23].

Consequently, microalgae remediation is a promising biological and environmentally friendly method of removing heavy metals from contaminated water. Their cell wall functional groups (e.g., carboxyl, hydroxyl, and amine groups) are mainly responsible for effective biosorption. Non-living algal biomass is especially efficient in passive adsorption and is therefore fit for extreme conditions, since it is resistant to metal toxicity and requires very low maintenance. This approach is considered as a single-use one, unless the biomass is subsequently processed for metal recovery [6]. In contrast, living microalgae offer dual functionality due to the complementarity of passive biosorption with metabolism-driven

uptake, allowing intracellular sequestration of metals and the regeneration of binding sites through growth [11]. This active method is enabled by photosynthesis and assists in removing metals while promoting water quality by the production of oxygen to counteract eutrophication and nurture aquatic life [24]. Apart from their bio-treatment possibilities, microalgae are highly prized for high-value co-products, such as biofuels [25–27], fertilizers [28], and in the food industry [29,30], including animal feed [31]. Following this, their recovery gives further evidence to circular economy perspectives [32,33]. Despite the challenges, such as sensitivity to high metal concentration and the need for special cultivation conditions, microalgae are a promising tool in heavy metal removal and restoring the ecosystem due to their adaptive features, multi-functionality, and sustainable technology [34].

Microalgae have developed various self-protection mechanisms to counteract the toxicity of heavy metals, which can be beneficial at low concentrations but harmful at high levels. These mechanisms include adsorption or bioaccumulation. Adsorption occurs when metal ions bind to the surface of microalgae cells through physical and/or chemical processes such as ion exchange and electrostatic interactions without their incorporation into the cellular structure. On the other hand, bioaccumulation is the active uptake of metal ions into the cells, where they can become integral parts of the cell's metabolic machinery, either by substituting for essential elements in enzymes or being stored in vacuoles for detoxification [1,7,14,35–37]. Chelation and complexation with active groups on cell walls, where negatively charged functional groups bind heavy metals, and ion exchange on cell walls, where ions like Na^+, K^+, Ca^{2+} and Mg^{2+} are replaced by toxic heavy metal ions through physical forces, also enhance metal binding. Additionally, extracellular polymeric substances (EPSs) enhance adsorption on the cell surface and prevent the accumulation of heavy metals within the cell. Microalgae also synthesize antioxidant enzymes, such as superoxide dismutase and catalase, to mitigate heavy metal stress. Heavy metals accumulate in cytoplasmic vacuoles, chloroplasts, and mitochondria, while biotransformation processes include reduction, methylation, and conversion from inorganic to organic states. These processes ensure the survival and growth of microalgae in environments with varying levels of metal toxicity [6,38].

Arthrospira platensis (*A. platensis*), formerly *Spirulina platensis*, is a resourceful microalga that is highly valued for its high protein, amino acid, vitamin, and mineral content, making it a popular supplement in the food, pharmaceutical, and cosmetic industries. One of its environmental applications is heavy metal removal, with its cell walls, rich in functional groups like carboxyl and phosphate, able to effectively bind metals. Dried *A. platensis* biomass is efficient in passive adsorption, while living cultures combine this with active uptake, sequestering metals intracellularly and improving water quality by releasing oxygen during photosynthesis [39]. This dual mechanism makes *A. platensis* effective for detoxifying industrial wastewater, mining effluents, and polluted water systems. Its eco-friendly nature and potential for biofuel and other valuable byproducts enhance its appeal for sustainable remediation [40].

Extensive studies were conducted regarding the bioremediation of single metal systems (mono-metal-treated, mono-MT), examining the processes, kinetics, and their removal efficiency [5,12,14,15,19,35,41–45]. These studies have provided valuable insights into the adsorption and biosorption capabilities of numerous microalgae. However, natural ecosystems often contain complex mixtures of metals, and as such, removal systems must be studied in multi-metal (multi-metal-treated, multi-MT) configurations. The research into the effectiveness of *A. platensis* bioremediation of multi-MT systems remains limited regarding the use of living cultures [36,46], and of dry *A. platensis* biomass [39]. Mixed metals often compete for binding sites, complicating the biosorption process. Variations

in metal properties, such as ionic radii and chemical behavior, further challenge multi-metal removal. Addressing these gaps is crucial for scaling algae-based bioremediation to industrial applications capable of handling real-world scenarios involving multiple contaminants [39]. *A. platensis* is considered as an effective biosorbent because of its large surface, polysaccharide content, and functional groups, allowing good coordination of the metallic cations. However, most research emphasizes single metal sorption performance, leading, subsequently, to the considerable underestimation of the realistic applications concerning multi-metal situations.

The scope of this study is to evaluate the ability of *A. platensis* in heavy metal remediation of multi-metal wastes. For this reason, microalga's cultures were exposed to mono- and multi-metal solutions of Cu, Cd, Ni, Pb, and Zn, and their metal adsorption ability was comparatively examined in every case. The heavy metal removal efficiency from *A. platensis* cultures was evaluated using atomic absorption spectroscopy (AAS). Additionally, the biomass, before and after remediation, was qualitatively examined using spectroscopic and microscopic methods to explore its potential use to other applications, while its growth estimation was performed using Near-Infrared Spectroscopy. These measurements were also performed to evaluate the changes induced by heavy metal stress.

2. Materials and Methods

2.1. Microalga Initial Stock Cultivation Conditions

The microalga studied was procured from HEALTHALGAE Sweden AB (Uppsala, Sweden). Its cultivation was performed using Spirulina Medium, as described in [47]. It is composed of four (4) stock solutions (Table 1) and the ingredients used were procured by Sigma-Aldrich (St. Louis, MO, USA), Carl Roth (Karlsruhe, Germany), Chem-Lab NV (Zedelgem, Belgium), and ACS Chemicals (Ahmedabad, India). An initial stock of *A. platensis* culture was axenically cultivated using the produced Spirulina Medium, with an initial pH of 9.4, constant aeriation of ~1 L/min, and illumination of ~160 μmol photons $m^{-2}s^{-1}$ of photosynthetically active radiation (PAR) in light/dark cycles of 12 h:12 h, using a full spectrum LED array (LUMATEK, ATTIS 200 W). Temperature was kept constant at 27 ± 0.5 °C using a water bath.

Table 1. The composition of the stock solutions used for the preparation of Spirulina Medium [47].

Stock Solution I (500 mL)		**Stock Solution II (500 mL)**		**Stock Solution III (900 mL)**		**Stock Solution IV (100 mL)**	
Component	**Amount**	**Component**	**Amount**	**Component**	**Amount**	**Component**	**Amount**
$NaHCO_3$	13.61 g	$NaNO_3$	2.50 g	$ZnSO_4 \cdot 7H_2O$	1 mL of 1 g/L	$FeSO_4 \cdot 7H_2O$	0.70 g
Na_2CO_3	4.03 g	K_2SO_4	1.00 g	$MnSO_4 \cdot 4H_2O$	2 mL of 1 g/L	Na_2-EDTA (Titriplex III)	0.40 g
K_2HPO_4	0.50 g	NaCl	1.00 g	H_3BO_3	5 mL of 2 g/L	Distilled water	
Distilled water		$MgSO_4 \cdot 7H_2O$	0.20 g	$Co(NO_3)_2 \cdot 6H_2O$	5 mL of 0.2 g/L		
		$CaCl_2 \cdot 2H_2O$	0.04 g	$Na_2MoO_4 \cdot 2H_2O$	5 mL of 0.2 g/L		
		$FeSO_4 \cdot 7H_2O$	0.01 g	$CuSO_4 \cdot 5H_2O$	1 mL of 0.005 g/L		
		Na_2-EDTA (Titriplex III)	0.08 g	Na_2-EDTA (Titriplex III)	0.40 g		
		SL III and SL IV	5 mL	Distilled water			
		Distilled water					

2.2. Mono- and Multi-MT Microalga Cultivation

The initial *A. platensis* stock culture, as described in Section 2.1, was centrifuged for 5 min at 5000 rpm, using a Multifuge X1 Pro-MD (Thermo Fisher Scientific, Waltham, MA, USA). The obtained biomass (~400 g, wet weight) was divided into 19 Erlenmeyer flasks of 500 mL.

The solutions of the heavy metals were prepared using the following nitrate salts of each metal in distilled water:

- Pb: $Pb(NO_3)_2$, Carl Roth.
- Cu: $Cu(NO_3)_2 \cdot 3H_2O$, Sigma-Aldrich.
- Zn: $Zn(NO_3)_2 \cdot 4H_2O$, Chem-Lab NV.
- Ni: $Ni(NO_3)_2 \cdot 6H_2O$, Sigma-Aldrich.
- Cd: $Cd(NO_3)_2 \cdot 4H_2O$, Thermo Scientific.

The mono-MT cultures were exposed to concentrations of 1, 5, and 10 ppm of each corresponding metal. Additionally, three flasks were prepared containing all metals in concentrations of 1 ppm (Mix 1), 5 ppm (Mix 5), and 10 ppm (Mix 10) of each metal for the multi-MT cultures. Finally, one flask was prepared without metal treatment (Control culture).

All Erlenmeyer flasks were filled with freshly prepared Spirulina Medium up to a total volume of 0.5 L for each culture. They were cultivated for 7 days with the same temperature, light, and aeration conditions as described in Section 2.1 [34]. Experiments were performed in duplicate (results are expressed as the respective averages).

The prepared cultures—as described in this section—were subsequently analyzed to monitor biomass growth and quality and metal removal efficiency. These methods are detailly described in the following sections (Sections 2.3.1–2.3.6).

2.3. Characterization Methods

2.3.1. Optical Microscopy

All cultures were monitored daily to observe the cultivation progress and the cells' states (shape, morphology) using an Olympus CX43RF microscope (Olympus Corporation, Tokyo, Japan). A 30 µL mechanically stirred culture sample of *A. platensis* was placed on a glass slide and covered with a cover slip. Photographs were captured with an Olympus EP50 digital camera (Olympus Corporation, Tokyo, Japan) using the microscope's 10×, 40×, and 60× objective lenses. An integrated LED source provided illumination for the samples.

2.3.2. Near-Infrared Spectroscopy (NIR)

Near-Infrared (NIR) Spectroscopy was employed as a rapid and easy method to estimate *A. platensis* biomass growth during its cultivation, following the protocol proposed by Malletzidou et al. [48]. Measurements were performed with a SPECTRUM Two N™ NIR spectrometer (Perkin Elmer, Waltham, MA, USA) using a diffused silica Near-Infrared Reflectance Module (NIRM), at 1000–2500 nm, with 8 cm^{-1} resolution and 64 scans. Measurements were performed daily without any sample preparation; 10 mL of cultivation were directly placed in a glass Petri dish (Ø 6 cm) without pretreatment, and they were returned to the culture after measurement, maintaining sterile conditions to avoid contamination. The diffuse reflectance NIR response at 1062.6 nm was used to depict the biomass content of *A. platensis* cultures. In order to minimize the impact of slight biomass variations among the 19 cultures under study, data were normalized to the ones collected on Day 0 of cultivation.

2.3.3. Electrical Conductivity (EC) and pH Measurements

To ensure optimal cultivation conditions, the pH and electrical conductivity (EC) of the cultures were monitored daily. The pH of the liquid media was measured directly using a pH-GL21 meter (Crison Instruments, Barcelona, Spain), with a resolution of 0.01 pH, calibrated with two buffer solutions (pH 4.01 and 7.01, HANNA Instruments, Leighton Buzzard, UK). EC was directly assessed with an Orion Star™ A212 electrical conductivity meter (Thermo Fisher Scientific, Waltham, MA, USA), with an accuracy of 0.5% of reading, calibrated with an EC 1413 μS/cm conductivity standard solution (HANNA instruments).

2.3.4. Chlorophyll Content Estimation

The chlorophyll-a content of the cultures was estimated with UV-Vis spectrophotometry as a cell health indicator [49]. For every culture, 4 mL were collected and centrifuged using a servo spin NECT centrifuge at 4000 rpm for 5 min. After the removal of the supernatant, 10 mL of methanol was added in the centrifugal tube; the latter—after using a vortex for 1 min—remained in a water bath at 90 °C for 10 min in an ultrasound device. Finally, the tube was again centrifuged for 5 min at 4000 rpm. The spectroscopic measurements were performed using a Lambda 25 spectrophotometer (Perkin Elmer, Waltham, MA, USA) with a scan speed of 60 nm/min, in the spectral area of 400–800 nm. Then, 3 mL from the supernatant were placed in a QG 10 mm cuvette for the measurement, while a second cuvette containing 3 mL of methanol was used as the "blank" sample.

A. platensis exhibits only chlorophyll-a, which has an absorption peak at 665.2 nm when using methanol as the extraction solvent [50]. For this reason, the absorbance at 665.2 nm was used for the estimation of the chlorophyll-a content, after the 750 nm reading subtraction, for turbidity correction reasons [51].

2.3.5. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectroscopy was applied to the dried biomass of the cultures, as collected on Days 0 and 7 of cultivation, to qualitatively monitor the microalga functional group changes induced by the metal interaction. The FTIR spectra were recorded with a Cary 670 spectroscope (Agilent Technologies Inc., Palo Alto, CA, USA) using a diamond attenuated total reflectance (ATR) accessory (GladiATR, PIKE Technologies, Madison, WI, USA). The spectra were collected in the mid IR area (4000–400 cm^{-1}), with 32 scans and a resolution of 4 cm^{-1}.

Micro-ATR-FTIR measurements using a focal plane array (FPA) detector were applied to the Control sample on Day 0 to inspect the spatial distribution of its components. This method was applied over standard ATR-FTIR measurements to provide high resolution chemical mapping at the microscale of single *A. platensis* filaments. For this reason, a single drop of the culture was placed on a glass slide and left to dry in room conditions (~25 °C). The measurements were performed with a Cary 620 FTIR microscope (Agilent Technologies Inc., Palo Alto, CA, USA)—coupled to the aforementioned FTIR spectroscope—equipped with a germanium micro-ATR accessory attached to a 15× objective visible/IR lens, using a 64 × 64 focal plane array (FPA) detector. Each FPA image consists of 4096 data points/individual spectra, each one of them collected from a 1.1 × 1.1 μm^2 independent observed area. The measurements were performed in the range of 3950–700 cm^{-1}, with a resolution of 4 cm^{-1} and 64 co-added scans.

2.3.6. Atomic Absorption Spectroscopy (AAS)

The metals' residual concentrations in each culture medium were determined on Days 3 and 7 by an atomic absorption spectrophotometer (AAnalyst 800, Perkin–Elmer, Waltham, MA, USA) and the total percentage removal was calculated (compared to the

initial concentration at Day 0, also determined with AAS). A total of 10 mL from each culture was meticulously extracted to remove as few cells as possible and centrifuged for 5 min at 5000 rpm. The supernatant liquid was then removed and transferred to the AAS system. The instrument was set up for the analysis of the target heavy metals, using the appropriate wavelength for each metal (213.9 nm for Zn, 283.3 nm for Pb, 324.8 nm for Cu, 232.0 nm for Ni, and 228.8 nm for Cd). The flame (air–acetylene) was adjusted to ensure optimal atomization of the sample for accurate detection. All measurements were performed in triplicate and the results of each concentration were expressed as the respective averages.

3. Results and Discussion

3.1. Optical Microscopy

The microscopic images of the Control and the metal-treated cultures on Day 7 using a 10× objective lens are presented in Figure 1. The linear morphology of the filaments is noted, instead of a helical one. Although the morphology relates to cultivation conditions—especially with high illumination during laboratory or industrial cultivation [52]–this morphology of whole cultures of various *Arthrospira* (*Spirulina*) species was previously reported and discussed [53] (and referenced within). In brief, straight filaments are usually reported in laboratory conditions and this morphology has been assigned to mutations associated with cultivation conditions that are under stress, as the linear form presents greater survivability. Straight filaments were also reported in wild cultures [53], making this morphology widely known and accepted [54].

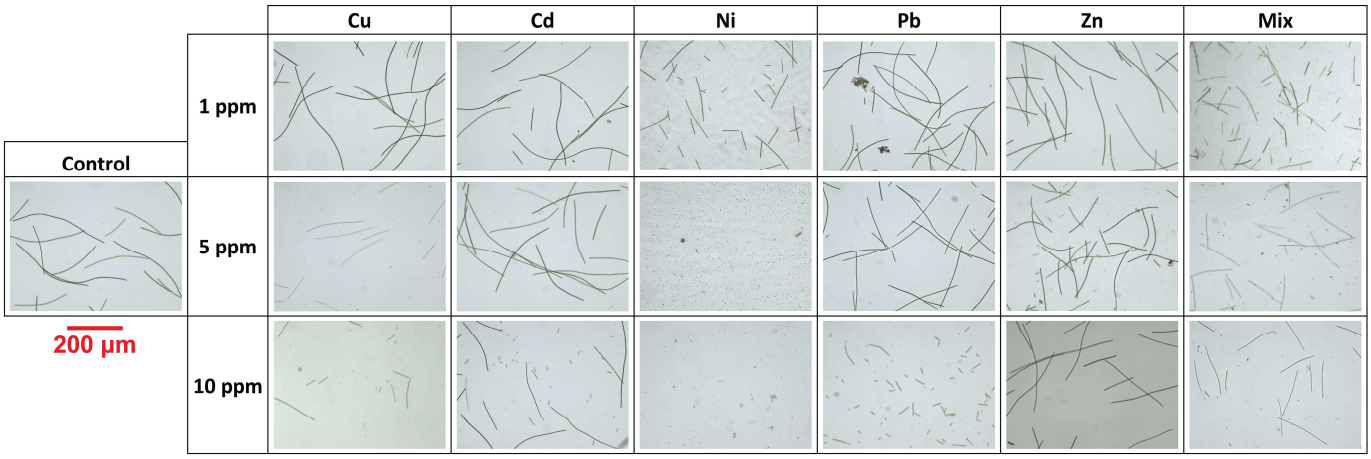


Figure 1. Optical microscopy images from all cultures as collected with ×10 objective lens on Day 7 of cultivation.

The images of Figure 1 (10× lens) and Figure 2 (60× lens) show the effect of the different metals (Cu, Cd, Ni, Pb, Zn) and their mixtures (Mix) on the cells in the three concentrations, in comparison with the Control culture [12]. The control cells have normal morphology (Figure 2a), while the treatment with metals, even at low concentrations (1 ppm), causes severe structural changes and reduced cell viability. The increase in concentration increases the cellular damage from 5 ppm to 10 ppm, with drastic changes taking place in the case of samples Ni, Pb, and Cu and the multi-MT Mix cultures. These include low cell density, fragmentation of cells (Figure 2b), structural and pigmentary degradation (Figure 2c), and possible cellular degeneration or the presence of protoplasts (Figure 2d). Therefore, the results highlight the toxicity induced by heavy metals, which is enhanced when combined.

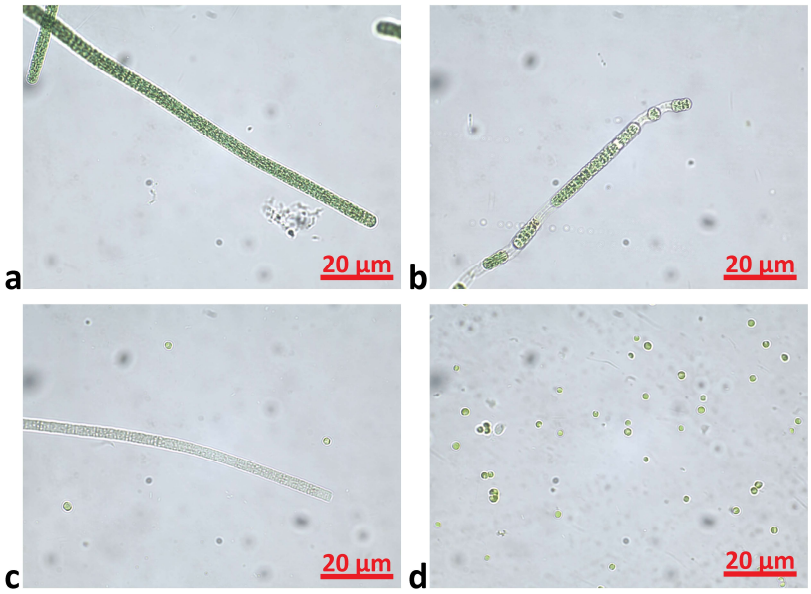


Figure 2. Representative optical microscopy images collected with ×60 objective lens on Day 7 of cultivation: (**a**) Control, (**b**) Mix 5 ppm, (**c**) Cu 10 ppm, and (**d**) Ni 5 ppm.

The changes observed include the loss of membrane integrity, which is manifested through disruption in the lipid bilayer, hence the uncontrolled flow and leakage of the cellular contents (Figure 2). Necridial cells occur due to the destruction of intercalary cells. This mechanism also leads to the fragmentation of the filaments that can lead to multiplication [53]. The cytoplasmic content becomes fragmented and the organelles and mitochondria swollen, thus indicating oxidative stress. Also, the nucleus usually appears deformed and shows chromatin condensation as a hallmark of apoptosis. Moreover, the cytoskeleton, which provides mechanical properties to the cell and transports intracellular materials, seems to disorganize to such an extent that cells lose their shape and become functionally impaired.

These structural changes agree with those in the literature [19], where it is stated that one of the primary targets for many toxicants is the cell membrane; the latter plays a vital role in maintaining cellular homeostasis and regulating the entry and exit of substances across the cells [53]. These membrane disruptions often promote organelle dysfunction, energy depletion, and eventually cell death.

3.2. Biomass Growth Estimation

Figure 3 shows the biomass growth of the cultures during the cultivation, using their NIR response at 1062.6 nm [48]. All measurements are normalized for direct comparison reasons, and the measurements of the Control culture are also presented for comparative reasons. The Control culture follows an exponential growth curve up to Day 5, followed by a decline phase, which agrees with the literature [29,53,55]. All Cd mono-MT cultures (Figure 3a) seem to be the lesser affected ones by the presence of the metals as they closely follow the same growth pattern with that of the Control culture [12]; it should be noted that the 10 ppm one presents a steeper decline phase. Zn mono-MT cultures (Figure 3b) also show similar characteristics with those of the Control culture. In this case, it should be noted that the 5 and 10 ppm Zn-treated cultures present a delayed exponential phase, never reaching a stationary plateau. The 10 ppm Pb mono-MT culture also presents a delayed exponential phase, while 1 and 5 ppm Pb mono-MT cultures follow the same characteristics with those of the Control culture, but with reduced spectral response/microalga biomass (Figure 3c) [19].

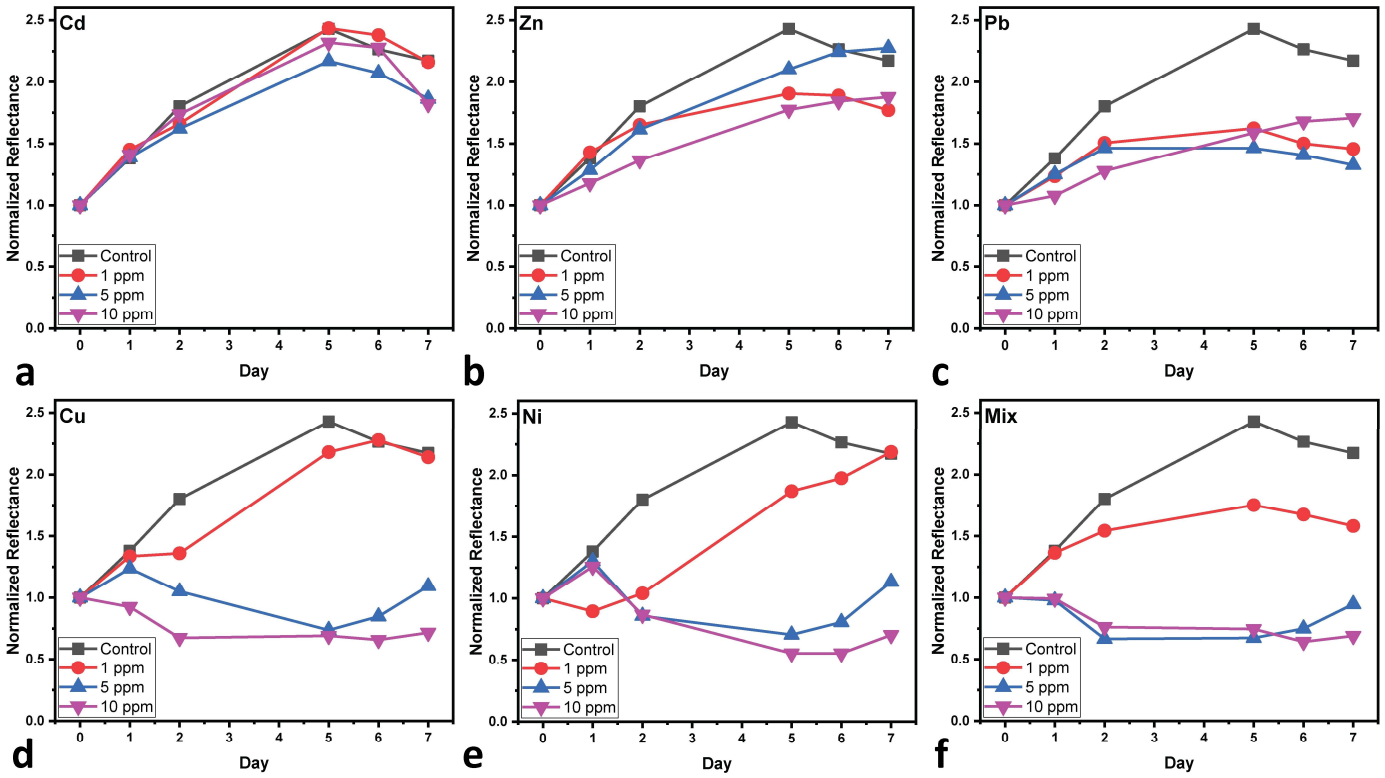


Figure 3. NIR reflectance response of cultures at 1062.6 nm: (**a**) Cd-MT; (**b**) Zn-MT; (**c**) Pb-MT; (**d**) Cu-MT; (**e**) Ni-MT; (**f**) Multi-MT. Values were normalized to Day 0 of cultivation to account for slight variations in initial biomass. Each value is average of two measurements, with uncertainties less than 3% over each value.

Cu and Ni mono-MT cultures (Figures 3d and 3e, respectively) are the most affected by the presence of metals [19]. In the case of Cu, the 1 ppm culture seems to be affected after Day 1 by the metal, but it follows the Control culture after Day 5 of cultivation. On the other hand, the 10 ppm culture seems to immediately decline, while the 5 ppm one starts to present an exponential phase after a decline period. Similarly, the 1 ppm Ni culture declines immediately, and it follows an exponential phase after Day 1 of cultivation, while the 5 and 10 ppm cultures start to decline after Day 1, with an exponential phase on the last day of cultivation. It should be noted that the 1 ppm Ni-treated culture biomass has an increasing tendency and slightly surpasses the Control culture. This behavior for the 1 ppm Ni-treated culture was reported in the literature [41].

All results indicate that microalga biomass growth is affected not only by the kind of metal but also by its concentration [15]. In the case of the multi-MT cultures, the 1 ppm culture presents an exponential phase, followed by a decline; the reduced reflectance values correspond to the reduced microalga biomass. Finally, the 5 and 10 ppm cultures quickly enter a decline phase. In this case, the 5 ppm culture begins to enter a growth phase after Day 5 of cultivation.

3.3. Electrical Conductivity and pH Measurements

EC monitoring during the cultivation of *A. platensis* is an important indicator of metabolic activity that affects culture health. Conductivity measures the medium's ability to conduct an electric current, which is influenced by ion concentration. During growth, the microalga absorbs the nutrient components such as nitrates (NO_3^-) and phosphates (PO_4^{3-}) from the cultivation medium, which are then integrated into the cells and thus reduce the ion concentration, reducing conductivity. However, in response to stressors such as metal exposure or nutrient limitation, metabolic processes of cells are disrupted

and the production of metabolic byproducts like hydrogen ions (H^+) or ammonium ions (NH_4^+) could increase, thus increasing EC. Increased conductivity may therefore become an indication of metabolic disturbances or culture stress [55]. Besides this, changes in conductivity can serve to monitor the general health and growth of the culture.

In stressed *A. platensis* cells, conductivity remains stable or slightly increases due to reduced metabolic activity and limited nutrient absorption. On the other hand, in healthy cells, conductivity decreases due to the absorption of nutrients by the cells, reducing ion concentration in the medium. In all cases, EC started with a value of 0.91 mS/cm for all cultures, while on Day 7, a minor variation is observed (<5% of the initial value).

In Figure 4, the pH measurements of all mono- and multi-MT cultures are presented, along with those of the Control culture, for all days of cultivation. In general, the measurements are summarized as follows:

- Zn, Pb, and Cd mono-MT cultures follow the Control one closely, at least up to Day 6.
- Ni and Cu mono-MT cultures present similar behavior.
- Regarding the multi-MT cultures, Mix 1 ppm seems to be unaffected up to Day 2 of cultivation. Afterwards, it starts declining. Mix 5 and 10 ppm cultures present reduced values even from Day 1.

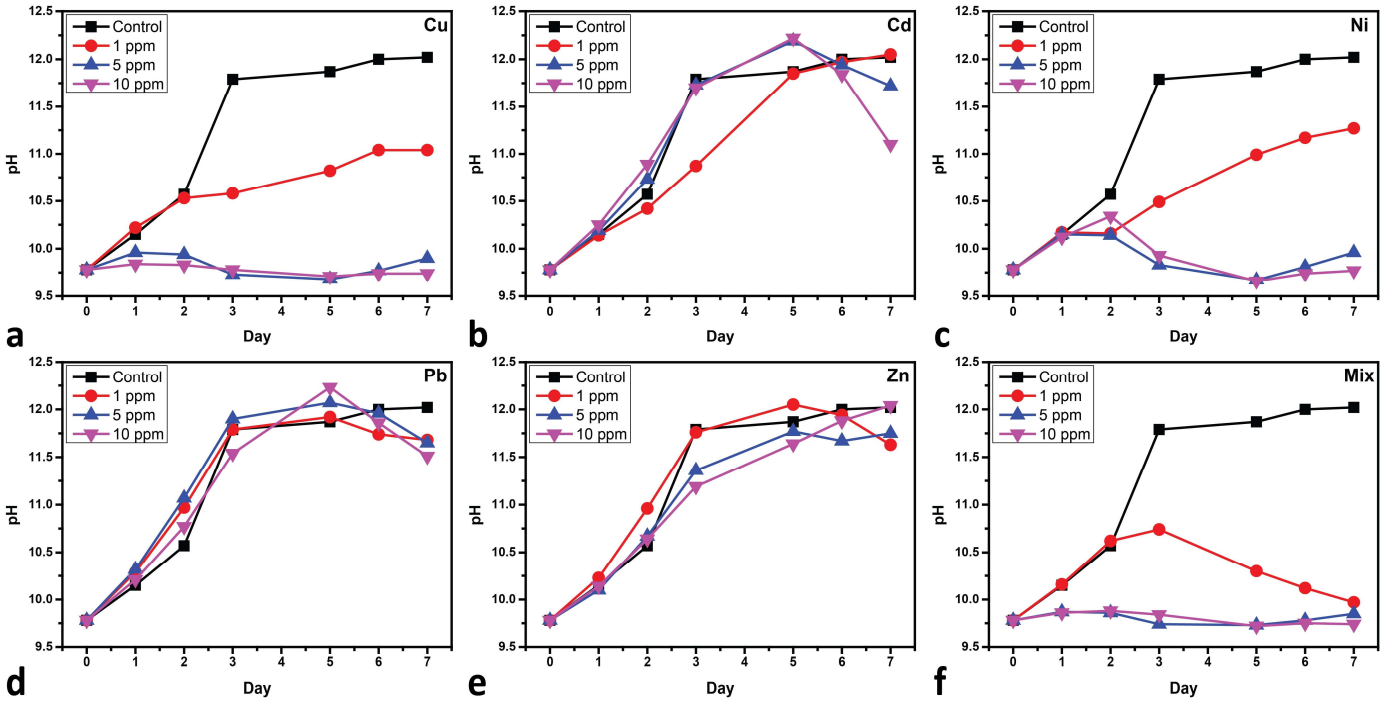


Figure 4. pH measurements of cultures during all cultivation days: (**a**) Cu-MT; (**b**) Cd-MT; (**c**) Ni-MT; (**d**) Pb-MT; (**e**) Zn-MT; (**f**) Multi-MT. Each value is average of two measurements, with uncertainties less than 4% over each value.

The increase in pH during *A. platensis* cultivation is a common phenomenon associated with the organism's photosynthetic activity and metabolic processes [19]. Optimal pH values for the microalga cultivation are between 9.5 and 11 [19]. *A. platensis* requires CO_2 in photosynthesis, and this diminishes the concentration of CO_2 in the medium. The carbonate equilibrium is moved, which increases alkalinity and pH. Besides this, the consumption of protons due to NO_3^- taken up as a nitrogen source increases the pH. These processes are balanced by the excretion of metabolic end products, such as $CaCO_3$, especially in hard water systems. Such pH increases are typical for healthy *A. platensis* growth and provide an alkaline environment that suppresses the growth of competitive

microorganisms. According to Vonshak [55], this interrelationship between photosynthetic CO_2 uptake and medium pH in *A. platensis* cultivation has a dual importance for the optimization of growth and prevention of contamination. Indeed, in our experiments, a clear difference in pH behavior was noticeable between the unstressed Control and the stressed MT cultures of *A. platensis*. Whereas in the normally growing samples, the pH showed a gradual increase during the time course, reflecting the photosynthetic and metabolizing activity in the strongly affected cultures—for example, the pH for the Ni-treated culture, as indicated by its morphology and biomass growth (Figures 1, 2d and 3e), remained unchanged throughout the cultivation period. The stress caused by the metal exposure may have shut off or drastically slow the metabolic activity of the cells, thereby preventing any noticeable pH increase. Such a response points to the susceptibility of *A. platensis* toward environmental stressors and further supports the use of pH changes as a rapid indicator of healthy versus stressed cultures.

3.4. Chlorophyll-A Estimation

A. platensis has only chlorophyll type "a" content [56,57]. It is a qualitative factor of *A. platensis* cultures as it depends on cultivation characteristics such as light, the medium of cultivation, the age of the cells, and the nitrogen concentration [58]. Chlorophyll-a in *A. platensis* is located within the thylakoid lamellae, which are membrane-bound structures in the cytoplasm. The thylakoid membranes are very important for the photosynthetic process of the organism since light energy is absorbed by chlorophyll and converted into chemical energy during photosynthesis. In these lamellae, chlorophyll is packed in such a way as to maximize light capture and enhance the efficiency of the photosynthetic process for *A. platensis* to flourish in different aquatic conditions and purify water [59].

The chlorophyll-a results (Figure 5) seem to support the quantitative and qualitative characteristics of the cultures, as presented from the previous measurements.

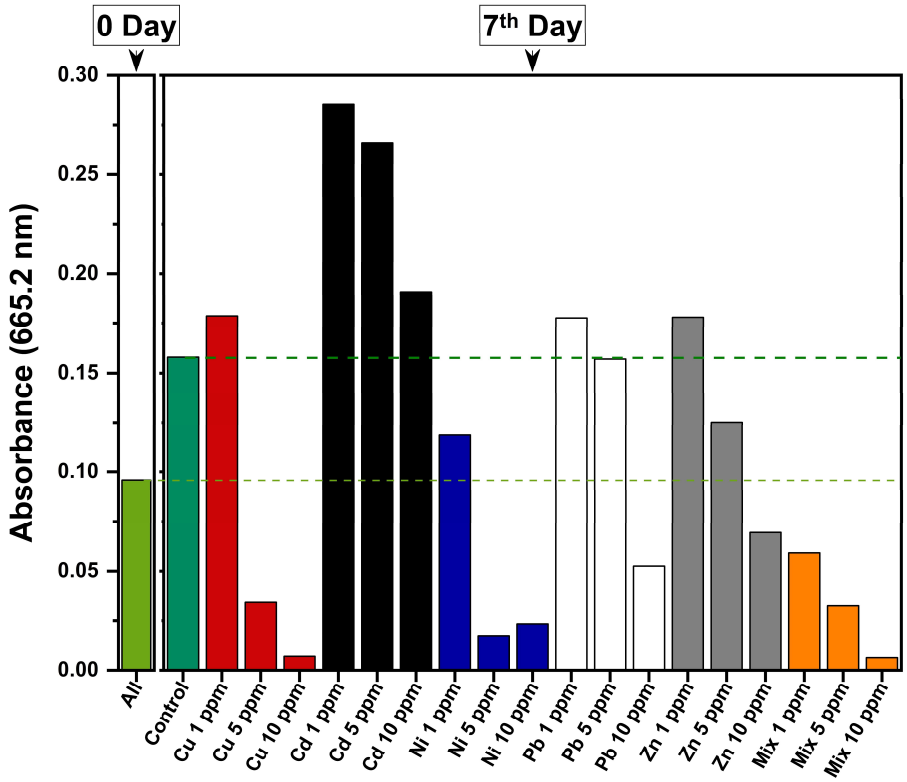


Figure 5. Chlorophyll-a content on Days 0 and 7 of cultivation of all cultures (Control and MT). Each value is average of two measurements, with uncertainties less than 5% over each one.

- Control culture: The chlorophyll-a increase relates to the biomass increase through cultivation.
- Cu and Ni mono-MT cultures: Low concentrations of 1 ppm seem to elevate the pigment's content, while higher concentrations exhibit toxic effects, drastically reducing it. This is in agreement with Kaamoush et al. [5], although we found similar toxicity of Ni in comparison to Cu. The toxicity of Cu to *A. platensis* relates to its role in increasing reactive oxygen groups. This affects lipids, proteins, and DNA, while it also substitutes Mg from chlorophyll. The authors [5] also showed that up to Day 7 of cultivation, low Ni concentrations, up to 1 ppm, act positively to the cultivations, surpassing the Control sample, while higher concentrations of Ni act negatively.
- Zn and Pb mono-MT cultures: Again, the low Zn concentration, that of 1 ppm, elevates the chlorophyll-a content. Kaamoush et al. [5] showed that up to Day 7 of cultivation, 1 ppm of Zn had similar results with their Control sample, but higher concentrations had a negative effect.
- Cd mono-MT cultures: All metal concentrations seem to elevate the chlorophyll-a content of the microalga, in comparison to the Control culture. This suggests an adaptive response and may be connected to the induction of stress-related metabolic pathways that affect growth. This hormetic response to low concentrations of Cd was observed with two other microalgae strains, *Spirulina indica* [60] and *Chromochloris zofingiensis* [61], and in peppermint plants [62].
- Multi-MT cultures: Their chlorophyll-a content reduces when metal concentration increases. While the 5 and 10 ppm cultures present similar characteristics with the mono-MT cultures, the Mix 1 ppm presents the lowest chlorophyll-a content, in comparison to all the mono-MT cultures.

It should be noted that the differences that seem to appear between Figures 3 and 5 are due to the applied methods. NIR spectroscopy directly estimates *A. platensis* biomass/overall growth. In contrast, chlorophyll-a content serves primarily as an indicator of cell health and photosynthetic activity. Under heavy metal stress, chlorophyll is a cell health indicator, and shows the damage of photosynthetic systems, before the detection of significant reductions in biomass. This is consistent with the microscopy observations (Figures 1 and 2), which show visible morphological changes and cellular stress despite a relatively stable biomass in some conditions.

The enhancement and the reduction in chlorophyll-a content of *A. platensis* upon heavy metal exposure can be attributed to several factors. The oxidative stress due to metals—such as Cu and Ni—results in the generation of reactive oxygen species (ROS), which are capable of cellular damage including chlorophyll and thylakoid membranes, thus resulting in the reduction in pigment content [5]. The hormetic effect also promotes chlorophyll synthesis, as mild stress triggers the onset of adaptive metabolic pathways due to low levels of metals. This eventually increases photosynthesis, while higher amounts disrupt photosynthetic machinery and thus disturb enzyme activities of chlorophyll synthesis [60,61]. The metals might also interfere with nutrient uptake, including magnesium, an integral constituent of chlorophyll, further inhibiting its production. In addition, detoxification mechanisms—such as metallothionein induction—may compete for substrates and other cellular resources necessary for chlorophyll biosynthesis during high-stress conditions of the cells [47]. Thus, while low metal concentrations may enhance chlorophyll-a, higher concentrations lead to a marked decrease on account of cellular damage and inhibited growth.

3.5. FTIR Analysis

ATR-FTIR analysis was performed on the *A. platensis* biomasses collected from all cultures as a qualitative method to check the changes occurring to microalga because of the

heavy metal influence. Figure 6 shows the ATR-FTIR spectra collected from the Control culture the initial (Day 0) and the final (Day 7) day of cultivation. Both spectra are almost identical, and they present FTIR bands that are typical for *A. platensis* [36,44,63–68]. In particular, the wide and intense overlapping bands at 3500–3200 cm^{-1} are attributed to OH and NH stretching vibrations, and the bands at 3000–2800 cm^{-1} are attributed to CH stretching vibrations [64,69]. The shoulder at 1728 cm^{-1} is attributed to C=O stretching of lipids [65]. The strong bands at 1643, 1541, and 1394 cm^{-1} are assigned to amide I (mainly C=O stretching), amide II (mainly N-H bending), and COO^- stretching, respectively. Some less intense bands and shoulders are also present at 1480–1180 cm^{-1}. In particular, these bands are located at 1451 (with many attributions to groups, such as OCH and COH [65], CH_3 [63,66,67], CH and CH_2 [36], and C-CH_3 and C-CH_2 [64]), 1308 (N-H bending and C-N stretching of amide III), and 1242 cm^{-1} (P=O of phospholipids and nucleic acids [63,68–70], and ester O-C-O [68]). Finally, the broad and intense complex at 1180–950 cm^{-1}, with defined bands at 1151, 1060, and 1029 cm^{-1}, is attributed to the saccharide content of the microalga (pyranose compounds), and especially to C-O and C-C bonds [36,65–69,71], and maybe with a contribution by P-O bonds [68,70].

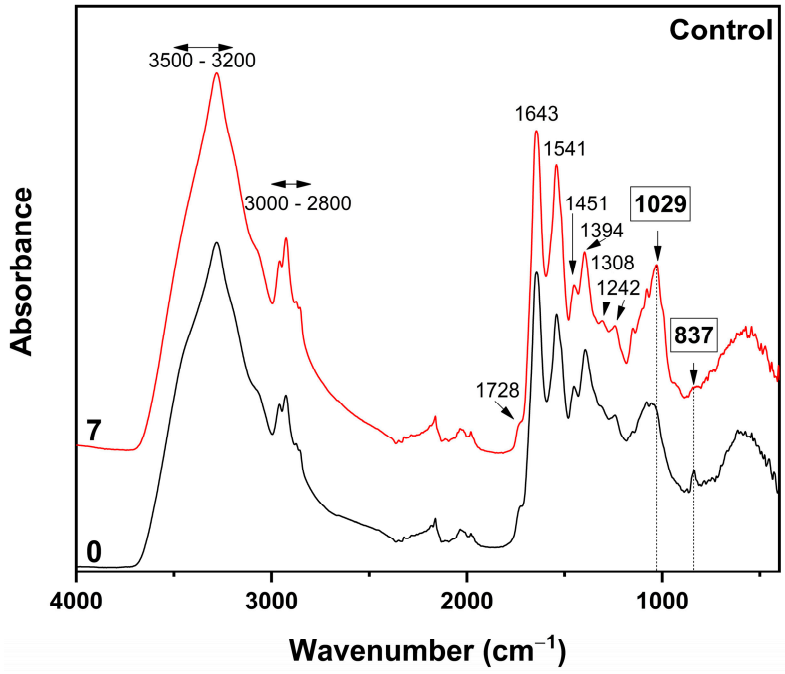


Figure 6. ATR-FTIR spectra of *A. platensis* biomass collected from Control culture on initial (Day 0) and final days (Day 7) of cultivation.

Regarding the variation in the Control culture spectra, a band at 837 cm^{-1} is present only in the Day 0 spectrum, while the area at 1180–950 cm^{-1} is stronger in Day 7, where the band at 1029 cm^{-1} is more intense. Both these variations are connected with saccharides [36,69,72–75].

The ATR-FTIR spectra of the biomass collected from all cultures on Day 7 of cultivation are presented in Figure 7. In every case, the main spectral differences between the MT cultures and the Control spectrum are noted for comparative reasons. The collected spectra show that the different metals cause changes in the microalga biomass. These differences can be summarized into two major categories: the decrease in the intensity of the 1643 (amide I) and 1541 (amide II) cm^{-1} bands, and the arise of bands attributed to saccharide content (~1400 cm^{-1} and 900–800 cm^{-1}). Similar results were also found in *A.*

platensis cultivations with different cultivation media and environmental conditions [22], and in mono- [45] and multi-MT cultivations [46].

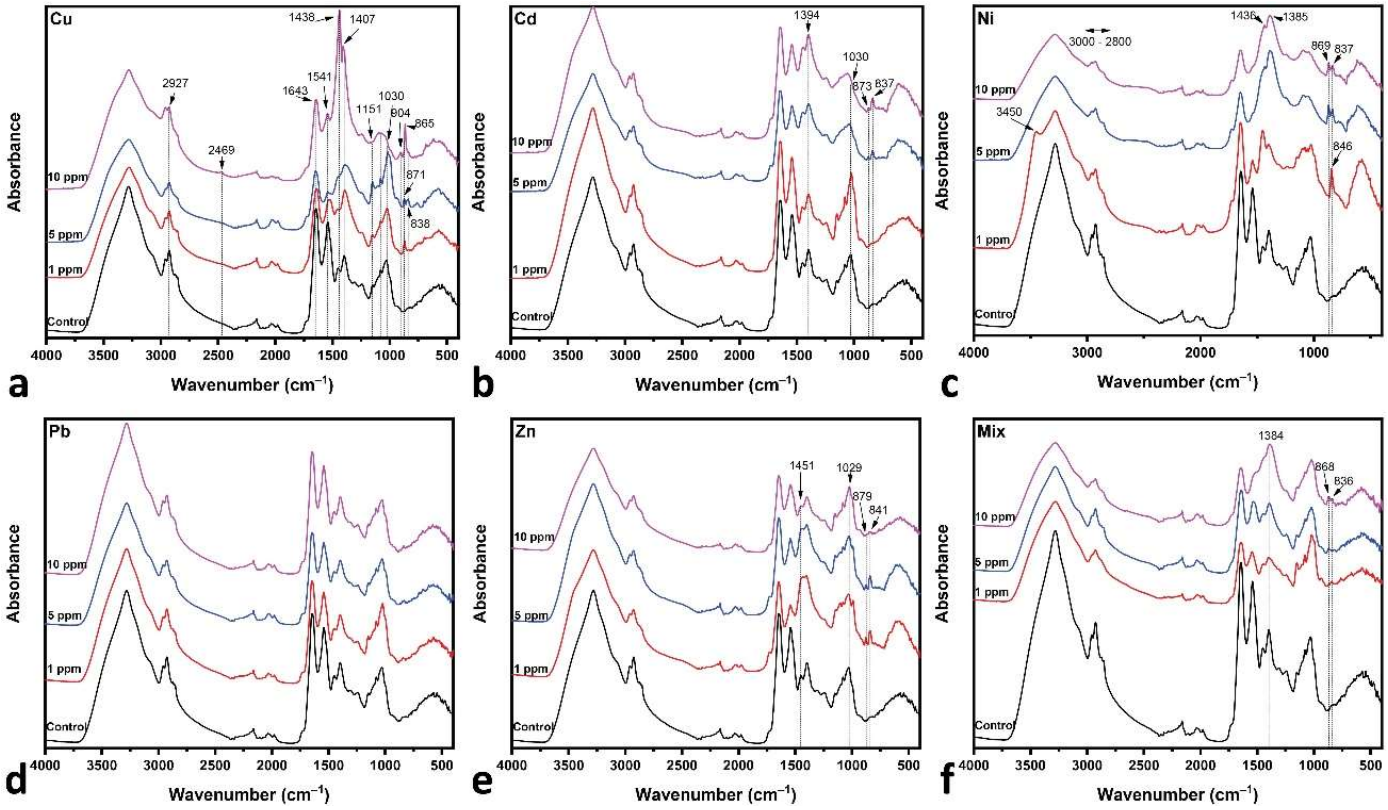


Figure 7. ATR-FTIR spectra of *A. platensis* biomass collected from all cultures on Day 7 of cultivation: (**a**) Cu-MT; (**b**) Cd-MT; (**c**) Ni-MT; (**d**) Pb-MT; (**e**) Zn-MT; (**f**) Multi-MT. Control culture spectrum is also shown for comparative reasons.

Concerning the adsorption of metals by cells, their cell walls and specifically the chemical groups present in them are considered responsible for binding metal ions [45]. Previous studies mention shifting of bands in the area of 1650–1300 cm^{-1}, which is indicative of metal binding to characteristic groups that present bands in this area, such as COO^- and OH groups [36,43,46]. In the present study, the shifting of bands in this area is not observed. Instead, the bands under question decrease in intensity in all MT cultures. This is indicative of the decline of these bands, i.e., the disruption of structure by the breaking of bonds, or the induction of oxidation stress leading to denaturation and fragmentation, up to the degradation of proteins, and not the coordination of their said functional groups with metals.

Regarding Cu, Zn, Cd, and Ni effects on *A. platensis* mono-MT biomass, similar spectral behavior was reported by [41]. The bands that arise in the 900–800 cm^{-1} spectral area are indicative of the prevalence of saccharides, and they are attributed to glycosidic bonds [74,75]. These bands are accompanied by the appearance of the bands at 1450–1400 cm^{-1} in the most affected cultures, such as Cu 10 ppm, Ni 5 and 10 ppm, and Mix 10 ppm, and they are again characteristic of saccharides [73,74]. This behavior was also reported regarding *A. platensis* cultures treated with HCl, presenting pH 14 [76], with the Cu-treated cultures [45], and also by polysaccharides extracted from *A. platensis* [71]. They are produced and accumulate on the cell wall to suppress the toxic effects from the metals as a cell defense mechanism. The metal ions are accumulated onto the cell walls by electrostatic interaction. This procedure is considered very rapid, and this step is followed by the transportation of the metals into the cytoplasm [1]. The accumulation of saccharides on the

cell walls is supported by the FPA analysis performed on the Control sample (Figure 8); the FPA chemical images of a single filament representing the spatial distribution of the bands at 1643 (C=O of amide I), 1728 (C=O of lipids), and 834 cm^{-1} (of saccharides) are presented. This confirms that the proteins are in the cell interior, while both the saccharide and lipid contents are mostly located in the cell walls. It should be mentioned that polysaccharides extracted from *A. platensis* have grown interest because of their biological properties against toxicity [75]. This cell defense mechanism—i.e., the accumulation of saccharides in the cell membrane—is further supported by the behavior of the Control sample on Day 0 of cultivation, as presented in Figure 6, where the emergence of the band at 837 cm^{-1} is noted, indicating that the microalga is stressed due to its adaption to the new environment caused by the addition of new cultivation medium.

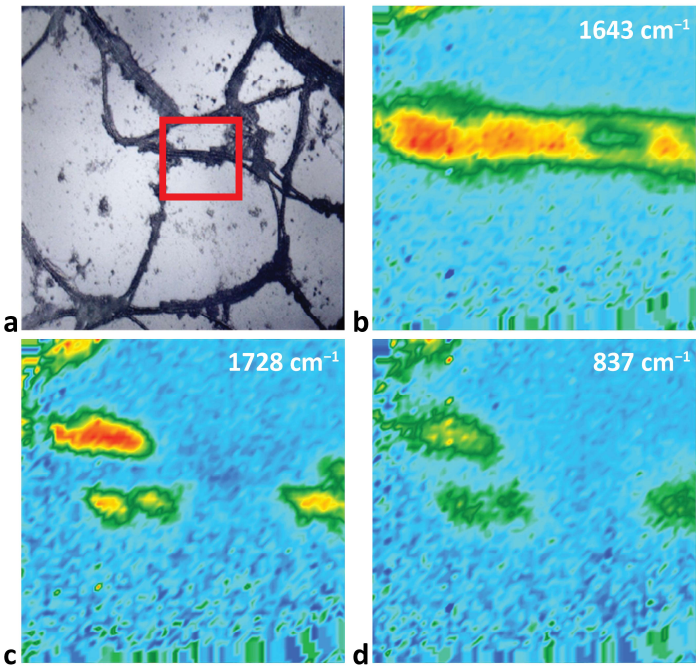


Figure 8. FPA/FTIR imaging analysis in micro-ATR mode of *A. platensis* filament (Control culture, Day 7 of cultivation). (**a**) Optical image (350 μm × 350 μm). Micro-ATR measured area is indicated with red rectangle (70 μm × 70 μm). FPA chemical images showing distribution of FTIR bands at (**b**) 1643, (**c**) 1728, and (**d**) 837 cm^{-1}, that correspond to amide I, lipids, and saccharides, respectively.

Finally, all Pb-treated cultures, and the Cd 1 ppm culture seem to be the least affected, after the cultivation period.

3.6. Heavy Metal Removal Efficiency of A. platensis in MT Cultures

Figure 9 shows the removal efficiency of *A. platensis* regarding mono- and multi-MT cultures. It was observed that as the concentration of Cu and Ni increases in a mono-component system, their toxicity becomes more pronounced at higher levels, potentially leading to lethal effects. However, at lower concentrations, Cu plays a vital role in the metabolic functions of both prokaryotic and eukaryotic organisms [18]. On the other hand, in multi-metal systems, Cu removal appears to increase compared to the mono-component system. When comparing the results between mono- and multi-metal systems, it is evident that Ni exhibits reduced removal in comparison to the other metals in the multi-MT cultures. This behavior can be explained by the competitive Langmuir model, which describes the interactions among various ions or molecules being adsorbed [2,45]. The model provides insights into the adsorption capacity in systems where multiple types of ions coexist, leading to competition for available adsorption sites. Low metal removal efficiency by

A. platensis is directly related to cell damage, as demonstrated in Figures 1, 2, 5, and 7, since cell integrity is crucial for metal binding effectiveness. As for the other metals, such as Cd, Pb, and Zn, their removal remains unaffected in the mono- and multi-component system. Furthermore, for these metals, higher concentrations could be reached without a noticeable decline in removal, suggesting that their uptake is not hindered by increased metal concentrations in the system [1,6].

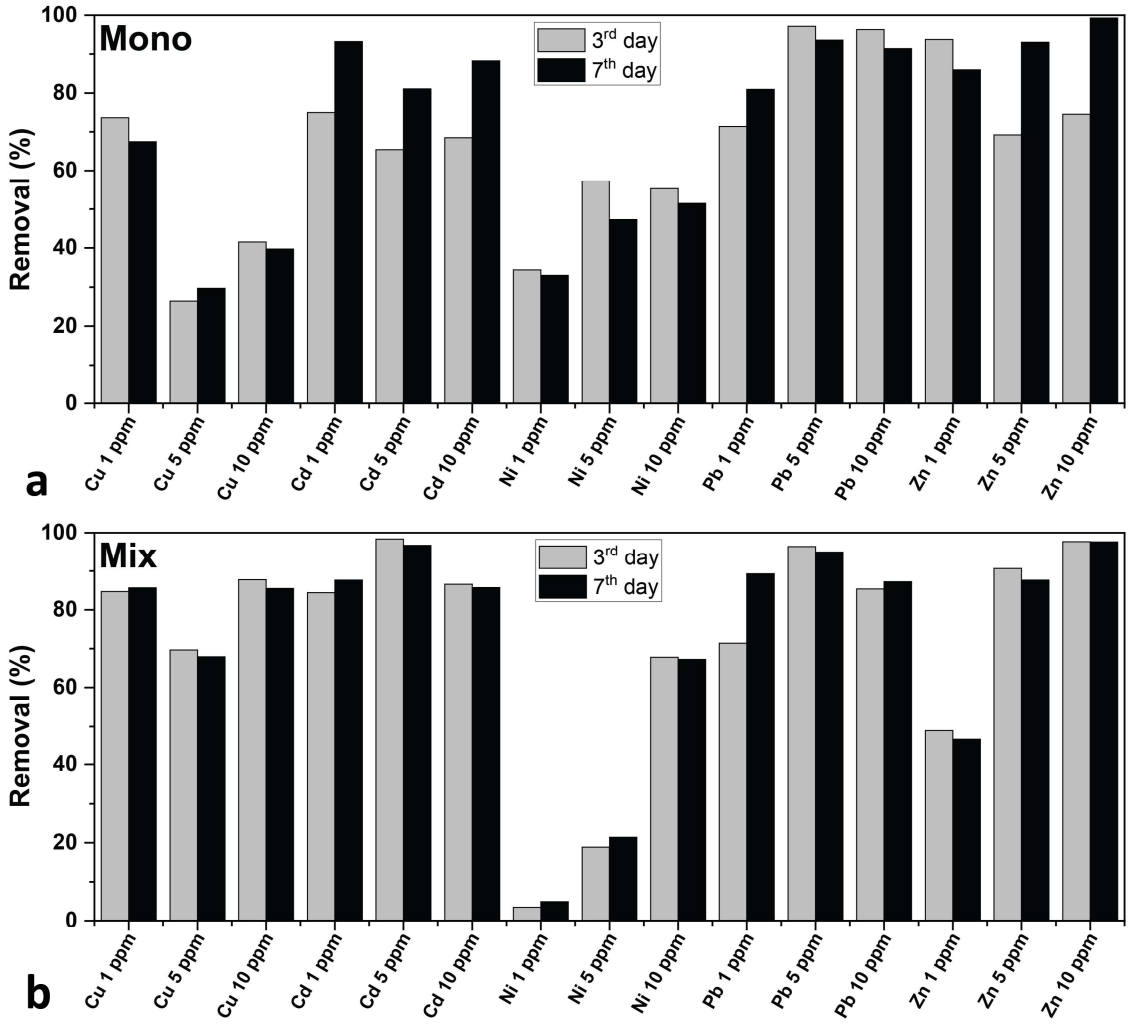


Figure 9. Removal (%) of heavy metals from culture media by *A. platensis* after mono- (**a**) and multi-metal (**b**) treatment of cultures. Each value is average of two measurements, with uncertainties less than 5%.

The primary mechanisms involved are surface adsorption, ion exchange, and chelation. Functional groups found in the cell walls, such as carboxyl, amino, and phosphate groups, attract and bind metal ions because of their negative charge, which draws in the positively charged heavy metal ions. Ion exchange enables the replacement of certain ions, like H^+ or Na^+, with metal ions, promoting their absorption. Additionally, metal ions can form complexes with organic molecules on the surface of *A. platensis* cells [1].

It is also observed that in some cases, heavy metal removal reduces on Day 7. This occurs primarily due to the reversible nature of the biosorption process where metal ions adhere to the surface of *A. platensis* cells but can be released when environmental conditions change. Factors such as pH, temperature, or the presence of competing ions can interfere with the binding sites, leading to the metals being freed [35]. When the microalga experiences stress due to excessive metal accumulation, it may actively expel excess ions

as part of its detoxification mechanisms to protect itself [77]. This is often facilitated by efflux pumps that transport metals out of the cell. Furthermore, prolonged metal stress can cause damage to the cell wall structure or lead to the instability of metal–ligand complexes inside the cells, which may result in the release of free metal ions into the environment [78]. Oxidative stress, a byproduct of heavy metal toxicity, can exacerbate cellular instability and disrupt intracellular storage mechanisms. These processes highlight the dynamic nature of metal removal efficiency of *A. platensis* and other microalgae. While they can accumulate and sequester metals, their ability to retain them is influenced by environmental factors and cellular stress, which can lead to the re-release of metals back into the surrounding environment when detoxification systems are overwhelmed [12,77].

Finally, all Cd and Zn 5 and 10 ppm mono-MT cultures present a delayed removal efficiency. Low Cd concentrations induce a hormetic effect [60,61], thus prompting proliferation over detoxification. On the other hand, Zn removal delay was previously reported [79]. This behavior was attributed to the transportation of the metal ions into the cell; the procedure is considered slower than surface adsorption [79]. This is also emphasized by the fact that both Zn and Cd have lower affinities for surface functional groups compared to the other metals of the present study [80–82].

4. Conclusions

In the present study, five different heavy metals were used to produce mono-MT and multi-MT cultures, to examine the behavior of multi-metal systems, and to compare the results regarding the ability of *A. platensis* for the bioremediation of water contaminated with heavy metals.

Experiments in multi-MT systems demonstrated that the removal of their components remains efficient compared to mono-MT systems, as particularly encouraging rates were achieved, i.e., 87% for Cu, 98% for Cd, 67% for Ni, 96% for Pb, and 97% for Zn, depending on the cultivation period and the concentration of heavy metals. The effect of Cu seems to be antagonistic to Ni. Regarding the simultaneous effect using different metals, their mixture may exhibit antagonistic or synergistic behavior, or it may show no interactive effects [39]. The results support that regarding the quality of the cultures and the cells themselves, Ni and Cu presented the most damaging effects, which is in agreement with the literature [19]. Cd, Pb, and Zn in mono-MT cultures had a moderate influence on cell viability in comparison with the damaging effects caused by Ni and Cu. This is supported by the literature in the case of Cd, Pb [19], and Zn. In the case of Cd, this finding is in contrast with the results of Diaconu et al. [19]; the authors, experimenting with Cd-, Ni-, and Pb-treated cultures, concluded that Cd had the most damaging result on the microalga.

The removal efficiency of *A. platensis*, considering the concentration of heavy metals and the day of cultivation, follows this order, in both mono- and multi-MT cultures, as presented in Table 2. In most cases, removals on Days 3 and 7 are quite close thus a shorter treatment period can be adopted. The removal mechanism that is induced by *A. platensis* can be considered as antagonistic over Ni 1 and 5 ppm- and Zn 1 ppm-treated cultures, as its removal efficiency decreased in comparison to the mono-MT culture. On the other hand, the removal mechanism is considered synergistic for all Cu, all Cd, Ni 10 ppm, all Pb, and Zn 5 and 10 ppm components of the multi-MT cultures, as the mix removal is elevated or close to the one of the mono-MT cultures.

Table 2. Removal efficiency order per metal for mono- and multi-MT cultures.

Concentration (ppm)	Metal Treatment	Day	Removal Efficiency Order
1	Mono	3	Zn > Pb > Cd > Cu > Ni
		7	Zn > Cd > Pb > Cu > Ni
	Multi	3	Cd = Cu > Pb > Zn > Ni
		7	Pb > Cd = Cu > Zn > Ni
5	Mono	3	Pb > Zn > Cd >Ni > Cu
		7	Pb = Zn > Cd > Ni > Cu
	Multi	3	Cd > Pb > Zn > Cu > Ni
		7	Cd = Pb > Zn > Cu > Ni
10	Mono	3	Pb > Zn > Cd > Ni > Cu
		7	Zn > Pb> Cd > Ni > Cu
	Multi	3	Zn > Pb > Cd > Ni > Cu
		7	Zn > Pb > Cd > Cu > Ni

The quality of the cultures was assessed by means of pH and EC measurements. EC measurements showed observed changes that did not exceed 5%; this is consistent with reduced metabolic activity and limited nutrient absorption from the cultivation medium. These small variations highlight that EC has limitations to be used as a standalone analysis to check cellular responses under the present study's conditions. On the other hand, pH measurements of the Cu-, Ni-, and multi-MT cultures of 5 and 10 ppm indicated that they may not be healthy. In particular, the multi-MT cultures (Mix) of 5 and 10 ppm show similar pH trends to those of Cu and Ni mono-MT cultures. The pH values of Mix 1 ppm presented a declining trend, indicating that this culture may also not be healthy, but the effect is delayed in comparison to the 5 and 10 ppm Mix cultures.

NIR spectroscopy was applied for biomass growth estimation. The Mix cultures' behaviors show similar results to those of the Cu-treated cultures.

Regarding the quality of the microalga cells, optical microscopy observations performed on Day 7 of cultivation show that the 1 ppm multi-MT culture seems to be the most affected, in comparison with the 1 ppm cultures of the mono-MT cultures. In contrast, the 5 and 10 ppm multi-MT cultures contain fragmented filaments, but they are not as affected as the Ni and Cu mono-MT cultures. Following this, the decrease in chlorophyll-a content of seemingly unhealthy cultures shows that the cells are not able to photosynthesize.

The collected ATR-FTIR spectra showed that the multi-MT cultures present characteristics that better agree with the Cu and Ni mono-MT cultures. The most affected ones are Cu 10 ppm, Ni 5 and 10 ppm, and Mix 10 ppm, as seen by the decline of the protein bands, while the saccharide- and lipid-associated bands seem to be unaffected. These results are confirmed from the optical microscopy images. For example, the Ni 5 and 10 ppm mono-MT cultures are degraded as no filaments—even broken—exist.

Based on the findings from the pH measurements and FTIR analysis, an insight into the metal uptake mechanisms can be partially provided. Both pH measurements and FTIR analysis suggest that bioaccumulation is involved. More specifically, the lower increase in pH of the stressed cells than that in the Control ones testifies to their probable impaired photosynthetic capacity. In relation to the latter, the FTIR data confirmed changes in cellular composition and thus further confirmed bioaccumulation. However, metal adsorption on cell surfaces is a mechanism that cannot be discarded.

Finally, based on the quality of the biomass after metal treatment, it is evident that it could be used for energy production using their saccharide content [13,63], and their polyunsaturated fatty acid content [13,63], for the production of biofuels, while the *A. platensis* polysaccharide content was reported for its use in bio-stimulants, as in material synthesis [71]. Biomasses used as a bio-adsorbent can be repurposed for sustainable applications in alignment with circular economy principles. Spent biomasses enriched with metals can be transformed into value-added products like catalysts, soil enhancers, and capacitors, or used for energy production in fuel cells or biochar synthesis, retaining adsorptive properties [33].

Based on the above, *A. platensis* appears to be a great candidate for the simultaneous bioremediation of heavy metal-contaminated waters, showing high growth rates and high removal efficiency for most metals. At the same time, the contaminated biomass has a high content in high-value substances that could be extracted and further used in various applications, supporting a green decontamination approach in the frame of circular economy.

Author Contributions: Conceptualization, N.A.K.; methodology, L.M., E.K., N.K., E.N., and N.A.K.; software, N.A.K.; validation, L.M., E.K., N.K., and N.A.K.; formal analysis, L.M., E.K., and N.K.; investigation, L.M., E.K., N.K., E.N., and N.A.K.; resources, N.A.K.; data curation, L.M., E.K., and N.K.; writing—original draft preparation, L.M., E.K., and N.K.; writing—review and editing, L.M., E.K., N.K., and N.A.K.; visualization, L.M.; supervision, N.A.K.; project administration, N.A.K.; funding acquisition, N.A.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research is financially supported under the research project "ALGEBRA—Innovative waste bioremediation practice for the removal of toxic compounds with the use of microalgae in the context of a circular economy", which is funded by the program "NATURAL ENVIRONMENT & INNOVATIVE ACTIONS 2022", PRIORITY AXIS 3: RESEARCH & IMPLEMENTATION, total budget: EUR 199,501.23, Green Fund, Athena—Research & Innovation Center in Information Communication & Knowledge Technologies.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

Acknowledgments: The authors would like to thank G. Vourlias for providing access to the FTIR facilities of the Laboratory of Advanced Materials and Devices (AMDE Lab), School of Physics, Aristotle University of Thessaloniki, Greece.

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

References

1. Kalita, N.; Baruah, P.P. Cyanobacteria as a Potent Platform for Heavy Metals Biosorption: Uptake, Responses and Removal Mechanisms. *J. Hazard. Mater. Adv.* **2023**, *11*, 100349. [CrossRef]
2. Mehta, S.K.; Gaur, J.P. Use of Algae for Removing Heavy Metal Ions from Wastewater: Progress and Prospects. *Crit. Rev. Biotechnol.* **2005**, *25*, 113–152. [CrossRef]
3. Xiao, X.; Li, W.; Jin, M.; Zhang, L.; Qin, L.; Geng, W. Responses and Tolerance Mechanisms of Microalgae to Heavy Metal Stress: A Review. *Mar. Environ. Res.* **2023**, *183*, 105805. [CrossRef]
4. Jayakumar, V.; Govindaradjane, S.; Rajamohan, N.; Rajasimman, M. Biosorption Potential of Brown Algae, Sargassum Polycystum, for the Removal of Toxic Metals, Cadmium and Zinc. *Environ. Sci. Pollut. Res.* **2022**, *29*, 41909–41922. [CrossRef]
5. Kaamoush, M.; El-Agawany, N.; El Salhin, H.; El-Zeiny, A. Monitoring Effect of Nickel, Copper, and Zinc on Growth and Photosynthetic Pigments of Spirulina Platensis with Suitability Investigation in Idku Lake. *Environ. Sci. Pollut. Res.* **2022**, *29*, 78942–78959. [CrossRef] [PubMed]

6. Suresh Kumar, K.; Dahms, H.U.; Won, E.J.; Lee, J.S.; Shin, K.H. Microalgae—A Promising Tool for Heavy Metal Remediation. *Ecotoxicol. Environ. Saf.* **2015**, *113*, 329–352. [CrossRef] [PubMed]
7. Arora, R. Adsorption of Heavy Metals-a Review. *Mater. Today Proc.* **2019**, *18*, 4745–4750. [CrossRef]
8. Fu, F.; Wang, Q. Removal of Heavy Metal Ions from Wastewaters: A Review. *J. Environ. Manag.* **2011**, *92*, 407–418. [CrossRef] [PubMed]
9. Bolto, B.; Gregory, J. Organic Polyelectrolytes in Water Treatment. *Water Res.* **2007**, *41*, 2301–2324. [CrossRef] [PubMed]
10. Van Der Bruggen, B.; Vandecasteele, C.; Gestel, T.V.; Doyenb, W.; Leysenb, R. Review of Pressure-Driven Membrane Processes. *Environ. Prog.* **2003**, *22*, 46–56. [CrossRef]
11. Wang, J.; Chen, C. Biosorbents for Heavy Metals Removal and Their Future. *Biotechnol. Adv.* **2009**, *27*, 195–226. [CrossRef] [PubMed]
12. Rangsayatorn, N.; Upatham, E.S.; Kruatrachue, M.; Pokethitiyook, P.; Lanza, G.R. Phytoremediation Potential of *Spirulina* (Arthrospira) *platensis*: Biosorption and Toxicity Studies of Cadmium. *Environ. Pollut.* **2002**, *119*, 45–53. [CrossRef] [PubMed]
13. Zhou, T.; Cao, L.; Zhang, Q.; Liu, Y.; Xiang, S.; Liu, T.; Ruan, R. Effect of Chlortetracycline on the Growth and Intracellular Components of Spirulina Platensis and Its Biodegradation Pathway. *J. Hazard. Mater.* **2021**, *413*, 125310. [CrossRef] [PubMed]
14. Zinicovscaia, I.; Cepoi, L.; Chiriac, T.; Ana Culicov, O.; Frontasyeva, M.; Pavlov, S.; Kirkesali, E.; Akshintsev, A.; Rodlovskaya, E. Spirulina Platensis as Biosorbent of Chromium and Nickel from Industrial Effluents. *Desalin. Water Treat.* **2016**, *57*, 11103–11110. [CrossRef]
15. Balaji, S.; Kalaivani, T.; Rajasekaran, C. Biosorption of Zinc and Nickel and Its Effect on Growth of Different Spirulina Strains. *CLEAN Soil Air Water* **2014**, *42*, 507–512. [CrossRef]
16. Soto-Ramírez, R.; Lobos, M.G.; Córdova, O.; Poirrier, P.; Chamy, R. Effect of Growth Conditions on Cell Wall Composition and Cadmium Adsorption in Chlorella Vulgaris: A New Approach to Biosorption Research. *J. Hazard. Mater.* **2021**, *411*, 125059. [CrossRef]
17. Edris, G.; Alhamed, Y.; Alzahrani, A. Biosorption of Cadmium and Lead from Aqueous Solutions by Chlorella Vulgaris Biomass: Equilibrium and Kinetic Study. *Arab. J. Sci. Eng.* **2014**, *39*, 87–93. [CrossRef]
18. Kiran Marella, T.; Saxena, A.; Tiwari, A. Diatom Mediated Heavy Metal Remediation: A Review. *Bioresour. Technol.* **2020**, *305*, 123068. [CrossRef] [PubMed]
19. Diaconu, M.; Soreanu, G.; Balan, C.D.; Buciscanu, I.I.; Maier, V.; Cretescu, I. Study of *Spirulina platensis* (Arthrospira) Development under the Heavy Metals Influence, as a Potential Promoter of Wastewater Remediation. *Water* **2023**, *15*, 3962. [CrossRef]
20. Hussain, M.K.; Khatoon, S.; Nizami, G.; Fatma, U.K.; Ali, M.; Singh, B.; Quraishi, A.; Assiri, M.A.; Ahamad, S.; Saquib, M. Unleashing the Power of Bio-Adsorbents: Efficient Heavy Metal Removal for Sustainable Water Purification. *J. Water Process Eng.* **2024**, *64*, 105705. [CrossRef]
21. Salama, E.S.; Roh, H.S.; Dev, S.; Khan, M.A.; Abou-Shanab, R.A.I.; Chang, S.W.; Jeon, B.H. Algae as a Green Technology for Heavy Metals Removal from Various Wastewater. *World J. Microbiol. Biotechnol.* **2019**, *35*, 75. [CrossRef] [PubMed]
22. Çelekli, A.; Topyürek, A.; Markou, G.; Bozkurt, H. A Multivariate Approach to Evaluate Biomass Production, Biochemical Composition and Stress Compounds of Spirulina Platensis Cultivated in Wastewater. *Appl. Biochem. Biotechnol.* **2016**, *180*, 728–739. [CrossRef]
23. Li, H.; Watson, J.; Zhang, Y.; Lu, H.; Liu, Z. Environment-Enhancing Process for Algal Wastewater Treatment, Heavy Metal Control and Hydrothermal Biofuel Production: A Critical Review. *Bioresour. Technol.* **2020**, *298*, 122421. [CrossRef]
24. Richmond, A. *Handbook of Microalgal Culture*; Blackwell Publishing Ltd.: Oxford, UK, 2013.
25. Baldev, E.; MubarakAli, D.; Sivasubramanian, V.; Pugazhendhi, A.; Thajuddin, N. Unveiling the Induced Lipid Production in Chlorella Vulgaris under Pulsed Magnetic Field Treatment. *Chemosphere* **2021**, *279*, 130673. [CrossRef] [PubMed]
26. Bayu, A.; Rachman, A.; Noerdjito, D.R.; Putra, M.Y.; Widayatno, W.B. High-Value Chemicals from Marine Diatoms: A Biorefinery Approach. *IOP Conf. Ser. Earth Environ. Sci.* **2020**, *460*, 012012. [CrossRef]
27. Xu, R.; Mi, Y. Simplifying the Process of Microalgal Biodiesel Production through in Situ Transesterification Technology. *JAOCS, J. Am. Oil Chem. Soc.* **2011**, *88*, 91–99. [CrossRef]
28. Spain, O.; Funk, C. Detailed Characterization of the Cell Wall Structure and Composition of Nordic Green Microalgae. *J. Agric. Food Chem.* **2022**, *70*, 9711–9721. [CrossRef] [PubMed]
29. Gabal, A.A.A.; Khaled, A.E.M.; El-Sayed, H.S.; Aboul-Ela, H.M.; Shalaby, O.K.; Khaled, A.A.; Mohamed, L.A. Optimization of Spirulina Platensis Biomass and Evaluation of Its Protective Effect Against Chromosomal Aberrations of Bone Marrow Cells. *Fish. Aquac. J.* **2018**, *10*, 260. [CrossRef]
30. Chen, H.B.; Wu, J.Y.; Wang, C.F.; Fu, C.C.; Shieh, C.J.; Chen, C.I.; Wang, C.Y.; Liu, Y.C. Modeling on Chlorophyll a and Phycocyanin Production by Spirulina Platensis under Various Light-Emitting Diodes. *Biochem. Eng. J.* **2010**, *53*, 52–56. [CrossRef]
31. Thiviyanathan, V.A.; Ker, P.J.; Hoon Tang, S.G.; Amin, E.P.; Yee, W.; Hannan, M.A.; Jamaludin, Z.; Nghiem, L.D.; Indra Mahlia, T.M. Microalgae Biomass and Biomolecule Quantification: Optical Techniques, Challenges and Prospects. *Renew. Sustain. Energy Rev.* **2024**, *189*, 113926. [CrossRef]

32. Singh, J.; Gu, S. Commercialization Potential of Microalgae for Biofuels Production. *Renew. Sustain. Energy Rev.* **2010**, *14*, 2596–2610. [CrossRef]
33. Kazakis, N.A. Green Approaches to Heavy Metal Removal from Wastewater: Microalgae Solutions in a Circular Economy Framework. *Soc. Impacts* **2025**, *5*, 100103. [CrossRef]
34. Saavedra, R.; Muñoz, R.; Taboada, M.E.; Vega, M.; Bolado, S. Comparative Uptake Study of Arsenic, Boron, Copper, Manganese and Zinc from Water by Different Green Microalgae. *Bioresour. Technol.* **2018**, *263*, 49–57. [CrossRef] [PubMed]
35. Ferreira, L.S.; Rodrigues, M.S.; de Carvalho, J.C.M.; Lodi, A.; Finocchio, E.; Perego, P.; Converti, A. Adsorption of Ni^{2+}, Zn^{2+} and Pb^{2+} onto Dry Biomass of Arthrospira (Spirulina) Platensis and Chlorella Vulgaris. I. Single Metal Systems. *Chem. Eng. J.* **2011**, *173*, 326–333. [CrossRef]
36. Dmytryk, A.; Saeid, A.; Chojnacka, K. Biosorption of Microelements by Spirulina: Towards Technology of Mineral Feed Supplements. *Sci. World J.* **2014**, *2014*, 356328. [CrossRef] [PubMed]
37. Çetinkaya Dönmez, G.; Aksu, Z.; Öztürk, A.; Kutsal, T. A Comparative Study on Heavy Metal Biosorption Characteristics of Some Algae. *Process Biochem.* **1999**, *34*, 885–892. [CrossRef]
38. Xu, K.; Du, M.; Yao, R.; Luo, J.; Chen, Z.; Li, C.; Lei, A.; Wang, J. Microalgae-Mediated Heavy Metal Removal in Wastewater Treatment: Mechanisms, Influencing Factors, and Novel Techniques. *Algal Res.* **2024**, *82*, 103645. [CrossRef]
39. Rodrigues, M.S.; Ferreira, L.S.; de Carvalho, J.C.M.; Lodi, A.; Finocchio, E.; Converti, A. Metal Biosorption onto Dry Biomass of Arthrospira (Spirulina) Platensis and Chlorella Vulgaris: Multi-Metal Systems. *J. Hazard. Mater.* **2012**, *217–218*, 246–255. [CrossRef] [PubMed]
40. Zeraatkar, A.K.; Ahmadzadeh, H.; Talebi, A.F.; Moheimani, N.R.; McHenry, M.P. Potential Use of Algae for Heavy Metal Bioremediation, a Critical Review. *J. Environ. Manag.* **2016**, *181*, 817–831. [CrossRef]
41. El-Agawany, N.; Kaamoush, M.; El Salhin, H. Nutritional and Toxicological Importance of Nickel, Copper and Zinc Elements in Spirulina Platensis. *Int. Marit. Transp. Logist. J.* **2023**, *12*, 55–77. [CrossRef]
42. Akbarnezhad, M.; Shamsaie Mehrgan, M.; Kamali, A.; Javaheri Baboli, M. Effects of Microelements (Fe, Cu, Zn) on Growth and Pigment Contents of *Arthrospira* (Spirulina) *platensis*. *Iran. J. Fish. Sci.* **2020**, *19*, 653–668. [CrossRef]
43. Gagrai, M.K.; Das, C.; Golder, A.K. Non-Ideal Metal Binding Model for Cr(III) Sorption Using Spirulina Platensis Biomass: Experimental and Theoretical Approach. *Can. J. Chem. Eng.* **2013**, *91*, 1904–1912. [CrossRef]
44. Fang, L.; Zhou, C.; Cai, P.; Chen, W.; Rong, X.; Dai, K.; Liang, W.; Gu, J.D.; Huang, Q. Binding Characteristics of Copper and Cadmium by Cyanobacterium Spirulina Platensis. *J. Hazard. Mater.* **2011**, *190*, 810–815. [CrossRef]
45. Çelekli, A.; Yavuzatmaca, M.; Bozkurt, H. An Eco-Friendly Process: Predictive Modelling of Copper Adsorption from Aqueous Solution on Spirulina Platensis. *J. Hazard. Mater.* **2010**, *173*, 123–129. [CrossRef]
46. Zinicovscaia, I.; Safonov, A.; Tregubova, V.; Ilin, V.; Cepoi, L.; Chiriac, T.; Rudi, L.; Frontasyeva, M.V. Uptake of Metals from Single and Multi-Component Systems by Spirulina Platensis Biomass. *Ecol. Chem. Eng. S* **2016**, *23*, 401–412. [CrossRef]
47. Spirulina Medium. CCCryo Cult. Collect. Cryophilic Algae 2020, 09/2020. Available online: https://utex.org/products/spirulina-medium (accessed on 19 March 2024).
48. Malletzidou, L.; Kyratzopoulou, E.; Kyzaki, N.; Nerantzis, E.; Kazakis, N.A. Near-Infrared Spectroscopy for Growth Estimation of Spirulina Platensis Cultures. *Methods Protoc.* **2024**, *7*, 91. [CrossRef]
49. Chapman, J.; Truong, V.K.; Elbourne, A.; Gangadoo, S.; Cheeseman, S.; Rajapaksha, P.; Latham, K.; Crawford, R.J.; Cozzolino, D. Combining Chemometrics and Sensors: Toward New Applications in Monitoring and Environmental Analysis. *Chem. Rev.* **2020**, *120*, 6048–6069. [CrossRef] [PubMed]
50. Lichtenthaler, H.K. Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes. *Methods Enzymol.* **1987**, *148*, 350–382. [CrossRef]
51. Parsons, T.R.; Strickland, J.D. Discussion of Spectrophotometric Determination of Marine-Plant Pigments, with Revised Equations for Ascertaining Chlorophylls and Carotenoids. *J. Mar. Res.* **1963**, *21*, 619. [CrossRef]
52. Zapata, D.; Arroyave, C.; Cardona, L.; Aristizábal, A.; Poschenrieder, C.; Llugany, M. Phytohormone Production and Morphology of Spirulina Platensis Grown in Dairy Wastewaters. *Algal Res.* **2021**, *59*, 102469. [CrossRef]
53. Sili, C.C.; Torzillo, G.; Vonshak, A. Arthrospira (Spirulina). In *Ecology of Cyanobacteria II, Their Diversity in Space and Time*; Springer: Dordrecht, The Netherlands, 2012; Volume 9789400738, pp. 677–705. ISBN 9789400738553.
54. Djearamane, S.; Lim, Y.M.; Wong, L.S.; Lee, P.F. Cytotoxic Effects of Zinc Oxide Nanoparticles on Cyanobacterium Spirulina (Arthrospira) Platensis. *PeerJ* **2018**, *6*, e4682. [CrossRef] [PubMed]
55. Vonshak, A. Spirulina: Growth, Physiology and Biochemistry. In *Spirulina Platensis (Arthrospira): Physiology, Cell-Biology and Biotechnology*; Vonshak, A., Ed.; Taylor & Francis: Abingdon, UK, 2002; pp. 43–66.
56. Águila-Carricondo, P.; de la Roche Cadavid, J.P.; Galán, P.L.; Bautista, L.F.; Vicente, G. New Green Biorefineries from Cyanobacterial-Microalgal Consortia: Production of Chlorophyll-Rich Extracts for the Cosmetic Industry and Sustainable Biogas. *J. Clean. Prod.* **2023**, *429*, 139652. [CrossRef]

57. Isar, M.; Cirik, S.; Turan, G. Production of Natural and Functional Pigments in Arthrospira (Spirulina) Platensis Cultivated in Laboratory Conditions. *Bull. Biotechnol.* **2022**, *3*, 11–15. [CrossRef]
58. Rangel-Yagui, C.D.O.; Danesi, E.D.G.; De Carvalho, J.C.M.; Sato, S. Chlorophyll Production from Spirulina Platensis: Cultivation with Urea Addition by Fed-Batch Process. *Bioresour. Technol.* **2004**, *92*, 133–141. [CrossRef] [PubMed]
59. Shevela, D.; Pishchalnikov, R.; Eichacker, L. Oxygenic Photosynthesis in Cyanobacteria. In *Stress Biology of Cyanobacteria: Molecular Mechanisms to Cellular Responses*; CRC Press: Boca Raton, FL, USA; Taylor & Francis Group: Abingdon, UK, 2013; pp. 3–40. [CrossRef]
60. Siva Kiran, R.R.; Madhu, G.M.; SV, S. Bioaccumulation of Cadmium in Blue Green Algae *Spirulina* (Arthrospira) Indica. *J. Bioremediation Biodegrad.* **2012**, *3*, 1–4. [CrossRef]
61. Zhang, Y.; Sun, D.; Gao, W.; Zhang, X.; Ye, W.; Zhang, Z. The Metabolic Mechanisms of Cd-Induced Hormesis in Photosynthetic Microalgae, Chromochloris Zofingiensis. *Sci. Total Environ.* **2024**, *912*, 168966. [CrossRef]
62. Wang, B.; Lin, L.; Yuan, X.; Zhu, Y.; Wang, Y.; Li, D.; He, J.; Xiao, Y. Low-Level Cadmium Exposure Induced Hormesis in Peppermint Young Plant by Constantly Activating Antioxidant Activity Based on Physiological and Transcriptomic Analyses. *Front. Plant Sci.* **2023**, *14*, 1088285. [CrossRef]
63. Manrich, A.; de Oliveira, J.E.; Martins, M.A.; Mattoso, L.H.C. Physicochemical and Thermal Characterization of the Spirulina Platensis. *J. Agric. Sci. Technol. B* **2020**, *10*, 298–307. [CrossRef]
64. Smith, B. *Infrared Spectrum Interpretation, a Systematic Approach*; CRC Press: Boca Raton, FL, USA, 1999; ISBN 0-8493-2463-7.
65. Bataller, B.G.; Capareda, S.C. A Rapid and Non-Destructive Method for Quantifying Biomolecules in Spirulina Platensis via Fourier Transform Infrared—Attenuated Total Reflectance Spectroscopy. *Algal Res.* **2018**, *32*, 341–352. [CrossRef]
66. Liu, H.J.; Xu, C.H.; Zhou, Q.; Wang, F.; Li, W.M.; Ha, Y.M.; Sun, S.Q. Analysis and Identification of Irradiated Spirulina Powder by a Three-Step Infrared Macro-Fingerprint Spectroscopy. *Radiat. Phys. Chem.* **2013**, *85*, 210–217. [CrossRef]
67. Kavitha, E.; Devaraj Stephen, L.; Brishti, F.H.; Karthikeyan, S. Two-Trace Two-Dimensional (2T2D) Correlation Infrared Spectral Analysis of Spirulina Platensis and Its Commercial Food Products Coupled with Chemometric Analysis. *J. Mol. Struct.* **2021**, *1244*, 130964. [CrossRef]
68. Biswas, A. Biosorption of Lead and Copper from Contaminated Solutions by the Cyanobacteria—Spirulina Maxima. Master's Thesis, California State University, Long Beach, CA, USA, 2017.
69. Socrates, G. *Infrared and Raman Characteristic Group Frequencies. Tables and Charts*, 3rd ed.; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2001; ISBN 978-0-470-09307-8.
70. Silverstein, R.M.; Webster, F.X.; Kiemle, D.J.; Bryce, D.L. *Spectrometric Identification of Organic Compounds*, 8th ed.; Wiley: Hoboken, NJ, USA, 2014; ISBN 9681308034.
71. Al-Badwy, A.H.; Khalil, A.M.; Bashal, A.H.; Kebeish, R. Polysaccharides from Spirulina Platensis (PSP): Promising Biostimulants for the Green Synthesis of Silver Nanoparticles and Their Potential Application in the Treatment of Cancer Tumors. *Microb. Cell Fact.* **2023**, *22*, 1–12. [CrossRef]
72. Tul'chinsky, V.M.; Zurabyan, S.E.; Asankozhoev, K.A.; Kogan, G.A.; Khorlin, A.Y. Study of the Infrared Spectra of Oligosaccharides in the Region 1000–40 cm^{-1}. *Carbohydr. Res.* **1976**, *51*, 1–8. [CrossRef]
73. Wang, Q.; Liu, F.; Chen, X.; Yang, Z.; Cao, Y. Effects of the Polysaccharide SPS-3-1 Purified from Spirulina on Barrier Integrity and Proliferation of Caco-2 Cells. *Int. J. Biol. Macromol.* **2020**, *163*, 279–287. [CrossRef] [PubMed]
74. Brezeștean, I.; Bocăneală, M.; Gherman, A.M.R.; Porav, S.A.; Kacsó, I.; Rakosy-Tican, E.; Dina, N.E. Spectroscopic Investigation of Exopolysaccharides Purified from Arthrospira Platensis Cultures as Potential Bioresources. *J. Mol. Struct.* **2021**, *1246*, 131228. [CrossRef]
75. Zhou, N.; Long, H.; Wang, C.; Zhu, Z.; Yu, L.; Yang, W.; Ren, X.; Liu, X. Characterization of Selenium-Containing Polysaccharide from Spirulina Platensis and Its Protective Role against Cd-Induced Toxicity. *Int. J. Biol. Macromol.* **2020**, *164*, 2465–2476. [CrossRef]
76. Chen, W.; Xu, J.; Yu, Q.; Yuan, Z.; Kong, X.; Sun, Y.; Wang, Z.; Zhuang, X.; Zhang, Y.; Guo, Y. Structural Insights Reveal the Effective Spirulina Platensis Cell Wall Dissociation Methods for Multi-Output Recovery. *Bioresour. Technol.* **2020**, *300*, 122628. [CrossRef]
77. Sytar, O.; Kumar, A.; Latowski, D.; Kuczynska, P.; Strzałka, K.; Prasad, M.N.V. Heavy Metal-Induced Oxidative Damage, Defense Reactions, and Detoxification Mechanisms in Plants. *Acta Physiol. Plant.* **2013**, *35*, 985–999. [CrossRef]
78. Wang, Y.; Ma, J.; Chu, H.; Zhou, X.; Zhang, Y. Advances in Microalgae-Based Livestock Wastewater Treatment: Mechanisms of Pollutants Removal, Effects of Inhibitory Components and Enhancement Strategies. *Chem. Eng. J.* **2024**, *483*, 149222. [CrossRef]
79. Nalimova, A.A.; Popova, V.V.; Tsoglin, L.N.; Pronina, N.A. The Effects of Copper and Zinc on Spirulina Platensis Growth and Heavy Metal Accumulation in Its Cells. *Russ. J. Plant Physiol.* **2005**, *52*, 229–234. [CrossRef]
80. Talapatra, N.; Mittal, V.; Maiti, T.K.; Ghosh, U.K. Algae-Based Biosorbent for Removal of Heavy Metals. In *Encyclopedia of Green Materials*; Baskar, C., Ramakrishna, S., Daniela La Rosa, A., Eds.; Springer: Singapore, 2023. [CrossRef]

81. Abbas, S.; Ismail, I.M.; Mostafa, T.M.; Sulaymon, A.H. Biosorption of Heavy Metals: A Review. *J. Chem. Sci. Technol.* **2014**, *3*, 74–102.
82. Ordóñez, J.I.; Cortés, S.; Maluenda, P.; Soto, I. Biosorption of Heavy Metals with Algae: Critical Review of Its Application in Real Effluents. *Sustainability* **2023**, *15*, 5521. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.