


## Article

# Application of *Salvinia biloba* Raddi. in the Phytoextraction of the Emerging Pollutant Octocrylene in an Aquatic Environment

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**Abstract:** The phytotreatment technique, which has never been used to treat emerging compounds, is used in this work to measure the phytoextraction of octocrylene (OC) in three concentrations (200, 400, and 600 µg/L of OC) by *Salvinia biloba* Raddi. The species proved to be a phytoextractor by accumulating OC in floating leaves at concentrations of 1,500,000 µg/kg in treatment 200 and 1,050,000 in 600 µg/L of OC. Chlorophyll synthesis was affected at all OC concentrations, especially 400 µg/L, with a chlorophyll *a/b* ratio of less than 1. Enzymatic activity responded to the contaminant: CAT and APX are inhibited in the submerged portions after 48 h, staying below  $2.0 \times 10^{-6}$  µmol/min/µg of protein. GPOX was totally inhibited during the experiment, and SOD remains active at 200 and 600 µg/L. The cytogenotoxic effects of OC to confirm phytoextraction were evaluated by globally regulated tests with *Allium cepa* bulbs and germinal bulbs in *Lactuca sativa* and *Avena fatua* every 48 h. These tests showed that after 72 h of phytoextraction, the medium was no longer cytogenotoxic and the seeds germinated above 30%, confirming the phytoextractor capacity of *S. biloba*. Thus, we can affirm that *S. biloba* can be used in the phytotreatment of aquatic environments contaminated with OC.

**Keywords:** phytotreatment; aquatic macrofite; cytogenotoxic



**Citation:** Moura, M.A.S.; Nascimento, G.C.S.G.; Valarini, O., Jr.; Peron, A.P.; Souza, D.C. Application of *Salvinia biloba* Raddi. in the Phytoextraction of the Emerging Pollutant Octocrylene in an Aquatic Environment. *Processes* **2024**, *12*, 1631. <https://doi.org/10.3390/pr12081631>

Academic Editor: Ibrahim M. Abu-Reidah

Received: 10 July 2024

Revised: 27 July 2024

Accepted: 31 July 2024

Published: 2 August 2024



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## 1. Introduction

The use and production of organic filters have increased absurdly in recent years. Some of these filters are lipophilic, with high Log Kow indicating their potential for accumulation in the biota [1]. It is estimated that the worldwide production of UV filters is above 10,000 tons [2,3]. Among the components of the filters, one of the main ones is octocrylene (OC), and its traces were observed mainly after 2010 in the biota components of different ecosystems [4,5]. Filters are part of various products ranging from cosmetics to textile industry stabilizers, paints, adhesives, plastics, and other polymers [6–8]. OC is an organic sunscreen from the cinnamate family; it acts on UV-B and UV-A shortwaves, making it widely used for this reason [5,9,10].

Studies reporting the accumulation of this UV filter in different ecosystems and its toxic effects have been disclosed, demonstrating that its effects are not restricted to places of use, such as beaches, but also due to the lack of adequate techniques for its elimination in wastewater treatment systems [11,12]. OC has been found in several organisms, such as algae, corals, fish, and mammals. The studies show that UV filters are not only absorbed but are transported through the food chain [4,13,14]. Due to their prolonged permanence in water and sediments, these compounds are transported by the current to different regions [2,15]. The estrogenic activity, genetic toxicity, and reproductive toxicity that OC causes in aquatic and plant species point to great concern regarding this filter [4,5,7,14,16].

Aquatic plants are important components of the aquatic ecosystem, serving as shelter and food. Because they absorb nutrients from sediments in the bed of rivers and lakes or from sediments near their root systems, they can significantly contribute to the dissemination of these compounds or can even act in the removal of these compounds from ecosystems if they are properly managed [17,18].

Studies of the phytoextraction capacity of several species have already been developed over the years, especially with the removal of metals from the environment [17–19]. However, there are no studies on the ability to extract and accumulate emerging substances, such as sunscreens. This fact highlights the importance of this study, which evaluates the survivability and phytoextractor capacity of *Salvinia biloba* Raddi in contact with OC. In addition, this type of treatment, when used appropriately, in addition to the efficiency of the species, presents itself as a technique with a low cost of implementation and maintenance. Thus, this study aims to evaluate the survivability of *S. biloba* in OC solution and the phytoextractive capacity of the substance in the environment.

It is worth noting that this study is a pioneer in the phytotreatment of emerging compounds with aquatic plants.

To achieve the main objective of the work, the following objectives were developed: (1) to evaluate the effects of the contaminant on the plant organisms to establish its phytoextraction capacity; (2) to determine the efficiency of the species *S. biloba* for this purpose; and (3) to verify the elimination of the effects of octocrylene through cytogenetic and phytotoxicity tests.

## 2. Material and Methods

### 2.1. Survivability and Phytoextraction of Octocrylene by *S. biloba*

#### 2.1.1. Obtaining, Defining, and Preparing OC Concentrations for Analyses

Octocrylene (2-ethylhexyl-2-cyano-3,3-diphenyl-2-propenoate, CAS 6197–30-4), as well as all other reagents used, were purchased from Sigma-Aldrich (Burlington, MA, USA) in analytical grade. The OC concentrations evaluated in the present study were defined based on quantification studies of this sunscreen in treated sewage sludge intended for the fertilization of cultivated soils and wastewater intended for irrigation. The concentrations were prepared in an aqueous medium using Tween 80 as a surfactant at a concentration of 0.214 g/L.

To check the stability of the sunscreen, OC was dissolved in Tween 80 (concentration: 0.214 g/L). The stability of OC was evaluated spectrophotometrically at 320 nm for millimolar concentration solutions with an initial absorbance (day zero) between 0.9 and 1.1 for a period of 15 days, during which stability was determined in percentage. For this, the absorbance value on day zero was considered 100%. The concentration values were measured using a calibration curve built using solutions of known OC concentrations. After 15 days of preparation, the OC stock solution remained stable, with a percent stability close to 100%.

#### 2.1.2. Experiment of Phytoextraction of OC by *S. biloba*

The treatment was carried out in three concentrations: 200 µg/L, 400 µg/L, and 600 µg/L of OC to test the survivability and extraction of the contaminant from the medium by *S. biloba*. The OC concentrations used are close to those found in the environment [4,7,15,20]. The species was chosen for its phytoextraction capacity from different contaminants, recognized

in different studies with aquatic phytoremediation [17,19]. The individuals used in the *S. biloba* experiment were kept under natural conditions in Clark's nutrient solution [21], free of environmental contamination, and cultivated for experiments at the Campus of the Federal University of Paraná, Campo Mourão.

Each container had three individuals of *S. biloba* and three containers for each withdrawal interval, making a total of forty-five individuals in each solution and control, with 180 individuals of *S. biloba* analyzed. The conditions of the experiment were light and room temperature (around 25 °C on the days of the experiment).

The test of survival and phytoextractor capacity was performed by 120 h of contact of the plants with the OC solutions. Individuals *S. biloba* plants were kept in plastic containers with a volume of 500 mL, each treatment was performed in triplicate and fifteen individuals were used per treatment, in addition to another 15 individuals were kept in containers with Clark's nutrient solution as control. Every 24 h, 200 mg of floating leaves and 50 mg of submerged leaves were removed and kept frozen at −20 °C for enzymatic analyses, and another 200 mg of floating leaves were also frozen for chlorophyll analyses.

The enzymatic analysis consisted of the cold maceration of 200 mg of floating portions and 50 mg of submerged portions in a mortar and pestle in a solution of 1 mL of 0.38% HCl and 2 mL of a 5 mM DTPA solution (diethylenetriaminopentaacetic acid). Subsequently, the samples were centrifuged at 13,000 rpm for 10 min. Of this enzyme extract, 150 µL was used in each enzymatic determination. The buffer used was potassium phosphate (0.5 M, pH 7.5), and the results were expressed in µmol/min/µg of protein for the enzymes catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPOX).

For the CAT, 1 mL of H<sub>2</sub>O<sub>2</sub> (0.0125 M) was added at the time of reading and read at 240 nm for one minute with an interval of two seconds. The activity was determined according to the decomposition of hydrogen peroxide and calculated by the extinction coefficient of 2.8 M ([22] with contributions from [23]).

APX had the addition of 0.1 mL of EDTA (0.5 mM), 0.5 mL of ascorbate (0.25 mM), and 1 mL of H<sub>2</sub>O<sub>2</sub> at the time of reading. Determined by the decrease of hydrogen peroxide in the reaction for one minute with an interval of two seconds at 290 nm, the extinction coefficient is 2.8 M [24].

GPOX was analyzed with a vortex mixture of 25 µL guaiacol (0.5%) and 25 µL H<sub>2</sub>O<sub>2</sub> solution (30%) for one minute. The solution was taken to an oven at 30 °C for 15 min, 25 µL of 2% sodium metabisulfite solution was added, and again vortexed, followed by a 10 min rest, read in a spectrophotometer at 450 nm, for one minute at two-second intervals. The extinction coefficient used for the calculation was 26.6 M [25].

The SOD was tested by the reaction of 0.5 mL of NBT (0.44 mM), 0.2 mL of riboflavin, and 0.5 mL of methionine (0.13 mM) with exposure to fluorescent light (80 W) for 20 min and a replica kept in the dark. The reading is at 560 nm, which calculates the difference between light and dark. The result expressed in U is the amount of enzyme required to inhibit 50% of NBT photoreduction [26].

The leaves collected for the determination of chlorophyll were frozen for two days in the total absence of light. Chlorophylls were determined with 200 mg of macerated leaves in 2 mL of an aqueous solution of 90% acetone in 10% magnesium carbonate saturation. Then it was centrifuged at 3000 rpm for 20 min and analyzed in a UV-VIS spectrophotometer at 664 nm and 647 nm by chlorophyll *a* and *b*, respectively [27].

The phytoextractive capacity of *S. biloba* was determined after 120 h of contact with the 200 µg/L, 400 µg/L, and 600 µg/L solutions of OC. The plants were removed and macerated with a pestle of 200 mg of floating portion and 50 mg of submerged portion of *S. biloba*. The samples were centrifuged at 10,000 rpm for 2 min (SOLAB/SL-702) and filtered on quantitative filter paper (Macherey-Nagel, Dueren, Germany) with a diameter of 20 µm. The filtered samples were measured with a UV-VIS spectrophotometer in the range of 305 nm [5]. All samples were taken in triplicate. From the calibration curve Absorbance = 0.01063 + 0.00336 OC (µg/L), the measured absorbance values were transformed into concentration.

## 2.2. Ecotoxicology of OC after Phytoextraction with *S. biloba*

To verify the efficiency of phytoextraction of *S. biloba*, toxicity analyses of the post-treatment solutions were carried out by means of the root meristems of *Allium cepa* and the germination potential in the cultivated species *Lactuca sativa* and in the spontaneous species *Avena fatua*. The species used to evaluate the efficiency of *S. biloba* are recommended by the OECD [28] for toxicity analysis of environmental samples and environmental pollutants from different sources. The *A. cepa* test has also been used to validate the efficiency of adsorbents in solutions in the presence of herbicides [29].

In this phase, the phytotoxicity, cytotoxicity, and genotoxicity of OC were evaluated by testing with *Allium cepa* L., a species internationally recognized in these tests. *A. cepa* was placed in contact with the solutions before any contact with *S. biloba* and in the solutions that had contact with *S. biloba* at intervals of 24, 72, and 120 h. For analysis of cytotoxicity and genotoxicity, five replications of bulbs were used for each treatment; these tests were performed according to Fiskejo [30].

Bulbs of *A. cepa* bulbs were purchased from an organic garden. Dried cataphylls were discarded, and bulbs were washed in distilled water. Then, the bulbs were placed in their respective treatments (control with distilled water or concentrations), remaining for five days (120 h) for root protrusion.

Phytotoxicity was evaluated based on root length. Ten roots of the root bundle of each bulb were measured with a digital caliper to determine the mean root length (RL) per treatment (Equation (1)). Other signs of toxicity were also considered, such as changes in the consistency and color of roots, the presence of tumors, hooked roots, and twisted roots.

$$\text{RL (cm): (Sum of root lengths of root bundles)/3} \quad (1)$$

For the evaluation of cytotoxicity and genotoxicity, roots were collected and fixed in Carnoy 3:1 (methanol:acetic acid) for 24 h. After fixation, roots were hydrolyzed in 1N HCl, and their meristem regions were excised to mount slides using the crushing technique, according to Herrero et al. [31]. Slides were analyzed under an optical microscope at 400× magnification.

Cytotoxicity was established based on meristem cell proliferation by counting cells in interphase, prophase, metaphase, anaphase, and telophase, and the Mitotic Index (MI) was calculated (Equation (2)), according to Santo et al. [5].

$$\text{MI: (Total number of dividing cells)/(Total number of cells analyzed)} \times 100 \quad (2)$$

Genotoxicity was defined based on the Cell Alteration Index (CAI), calculated from the number of cell alterations observed in meristems (Equation (3)), according to Santo et al. [5]. From each bulb, 300 cells were analyzed, totaling 900 cells per treatment. The categories of aberrant cells considered were micronucleus, viscosity, metaphase with chromosome breakdown, and abnormal ana/telophases, which include bridges, wandering chromosomes, chromosomal breakdown, and multipolar spindles.

$$\text{CAI: (Number of cellular changes)/900} \times 100 \quad (3)$$

The phytotoxicity test on seeds was according to the OECD [28]. The germination of seeds of *Lactuca sativa* L. cultivated species and the spontaneous *Avena fatua* L. used the solution after contact with *S. biloba* at 24-, 72-, and 120-h intervals. *A. fatua*, which represents native species, and *L. sativa*, which represents the cultivated species that would have contact with the contaminant in the soil, both, as indicated by the OECD, have also been used for Mañas and Heras [32]. Seeds of *A. fatua* and *L. sativa* were purchased from specialized stores, free of agrochemicals, and with a germination potential above 95%.

Seeds of similar size were separated. Before the experiments, seeds were sterilized in HgCl<sub>2</sub> (0.1%) for 1 min. After this, the seeds were washed three times in deionized water. Then, seeds from each plant were distributed in Petri dishes (20 seeds per dish), previously sterilized and lined with filter paper.

For treatment and control with distilled water, ten repetitions were made, totaling 200 seeds for the control and for each concentration. Immediately after preparing the concentrations, 1 mL of the treatments were placed on their respective dishes. Then, the dishes were wrapped in plastic film and immediately placed in a BOD (Biochemical Oxygen Demand) incubator at 25 °C, in the dark, for 120 h (five days). A seed was considered to have germinated when the radicle emerged.

The germination percentage (G) was determined as follows (Equation (4)), according to Biruk et al. [33]:

$$G(\%) : \frac{\text{number of seeds germinated}}{\text{number of seeds evaluated}} \times 100 \quad (4)$$

After five days, 10 roots of each dish were measured using a digital caliper to determine the Relative Growth Index (RGI) (Equation (5)) and the Germination Index (GI %) (Equation (6)) of treatments based on Biruk et al. [33]:

$$RGI = \frac{RLI}{RLC} \quad (5)$$

$$GI(\%) = \frac{RLI \times GSI}{RLC \times GSC} \times 100 \quad (6)$$

RLI is the average radicle length obtained after exposure to the tested concentrations, and RLC is the average radicle length obtained for the “control”. Therefore, GSI is the number of seeds germinated after exposure to the tested concentrations, and GSC is the number of seeds germinated in the “control”.

### 2.3. Statistical Analysis

The enzymatic activity data of CAT, APX, GOPX, and SOD and chlorophylls *a* and *b* were tested for normality by the Lilifors test; as they were not considered normal, the data were then analyzed by the Kruskal–Wallis [34] test (H test), and the significant data were applied by the Dun test ( $p \leq 0.05$ ) in BioEstat<sup>®</sup> software, version 5.3.

Data obtained in the phytotoxicity, cytotoxicity, and genotoxicity tests were tested for normality by the Lilifors test and for not being normal by the Kruskal–Wallis (H-test), followed by Dunn’s test ( $p \leq 0.05$ ) using the BioEstat<sup>®</sup> software, version 5.3. Cell viability results were plotted on a curve generated by non-linear regression. The results of mortality rates and the escape test were analyzed by Fisher’s and one-tailed Fisher’s exact tests, respectively, using the Action 6.2 software.

### 2.4. Main Materials Used for the Analyses

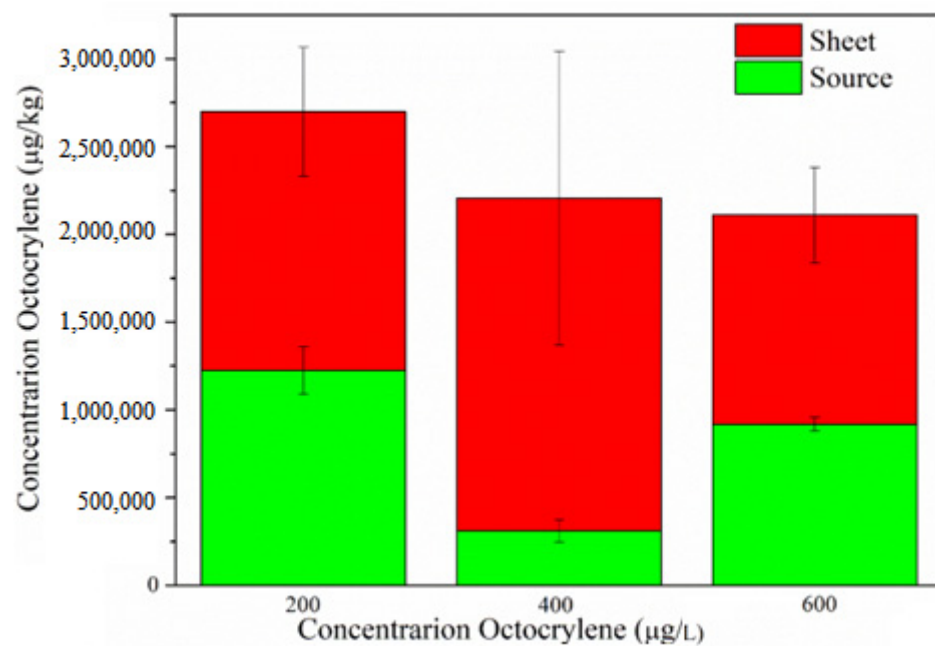
Utensils: beaker, Becker, graduated pipette and precision pipette, test tubes, slides and coverslips, spatula, Petri dishes, 500 mL polystyrene jars, grail with pestle, and liquid nitrogen. Equipment: scale, microscope, spectrophotometer, centrifuge, BOD (Biochemical Oxygen Demand) incubator, digital caliper.

## 3. Results and Discussion

After 120 h of exposure to the different treatments with OC, the analyses of the floating and submerged portions of *S. biloba* showed OC concentrations (Figure 1). To be considered phytoextractors, plants need to absorb the substance and transport it to different parts of the body [35]. The aerial parts of the plant contained the highest amount of organic sunscreen compared to the submerged ones (Figure 1), regardless of the concentration of OC.

At the 200 µg/L concentration, the accumulation of OC in the plant tissues was the highest, at about 1,500,000 µg/kg of plant, with a ratio of 0.83 between submerged and aerial portions. *S. biloba* transported to the aerial portions the highest amount at a concentration of 400 µg/L of OC with a ratio of 0.12. The concentration of 600 µg/L of OC showed an accumulation of 1,050,000 µg/kg with a ratio of 0.58 between the portions of the plant (Figure 1).



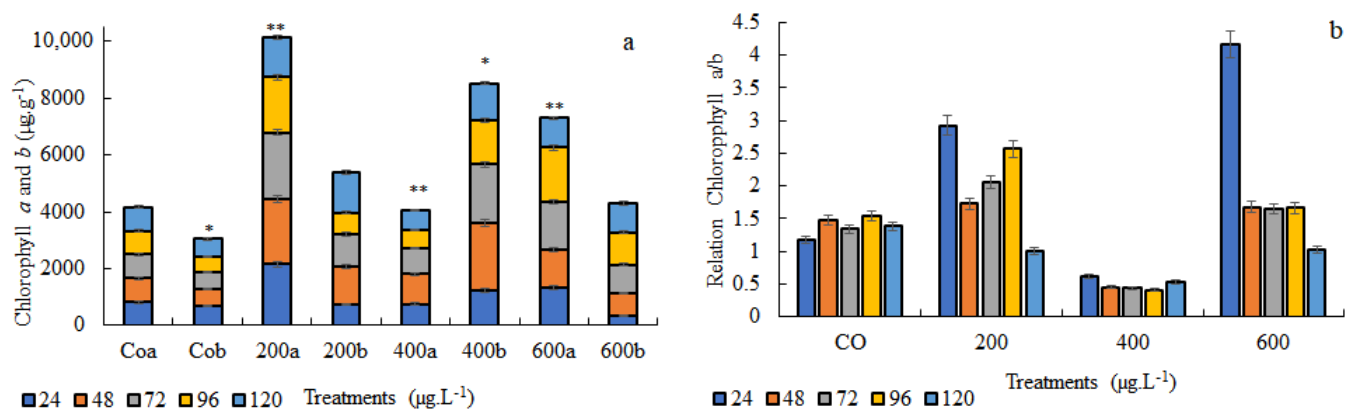


**Figure 1.** Concentration of Octocrylene (µg/L) in submerged and floating portions of *S. biloba*.

Although the highest accumulation was observed in the floating portions in total quantity, the plant accumulated more OC than the concentration of 200 µg/L of OC and showed a small difference between the concentrations of 400 and 600 µg/L of OC. However, it is important to highlight that the concentration of 400 µg/L of OC was the one that transported almost all the OC to the floating portions; this may indicate that the plants at this concentration had a higher affinity for the contaminant and may have caused the greatest failure of the defense system.

Considering the greater accumulation of OC observed in the aerial portions at all concentrations, we can state that *S. biloba* has phytoextractive capacity for this emerging pollutant. The process is considered phytoextraction when the contaminants are absorbed by the roots, transported, and accumulated in the aerial parts of the plant. Phytoextraction has been reported in the extraction of cadmium (Cd), lead (Pb), zinc (Zn), copper (Cu), chromium (Cr), nickel (Ni), selenium (Se), and mercury (Hg) from contaminated soils and waters [36], and can also be used for inorganic and organic compounds.

*S. biloba*, when absorbing and transporting OC from the submerged portions to the floating portions, it could show signs of senescence; however, morphological observations showed that after 120 h of experimentation, the plants did not appear to be physically damaged. Metabolic effects were observed in the synthesis of chlorophylls *a* and *b* (Figure 2), with a significant increase in chlorophyll *b* synthesis in the 400 µg/L OC treatment ( $H = 13.37$  and  $p < 0.03$ ). At a concentration of 200 µg/L of OC, there was the greatest increase in chlorophyll *a* production, followed by 600 µg/L of OC. At the 400 µg/L concentration of OC, the levels of chlorophyll *a* were below those of the other treatments but not in relation to the control (Figure 2a), which may corroborate the idea of the failure of the defense system to protect the plant organism at this concentration because, unlike the other treatments, there is almost no change in the concentration of chlorophyll *a* between the control and the other treatments ( $F = 12.78$ ,  $p < 0.001$ ). Chlorophyll *b* increased in concentration in all treatments, with fluctuations throughout the experiment period (Figure 2a). In the 400 µg/L OC treatment, the concentration of this chlorophyll increased from 607.37 µg/g to 1237.7 µg/g in the first 24 h and reached the highest concentration of 2378.65 µg/g in the 48 h of the experiment.



**Figure 2.** Chlorophyll *a* and *b* concentrations of *S. biloba* at concentrations of 200, 400, and 600  $\mu\text{g}/\text{L}$  Octocrylene for 120 h (5 days). \* or \*\* Indicate statistically significant differences in concentrations and control, according to Kruskal–Wallis, followed by Dunn’s test ( $p \leq 0.05$ ). Coa: chlorophyll *a* concentration of the control; Cob: chlorophyll *b* concentration of the control; (a): chlorophyll *a*; (b): chlorophyll *b*; CO: control.

The chlorophyll *a/b* ratio is generally 3:1, and *S. biloba* had a ratio of 1.5:1 in the control group (Figure 2b). There is an increase in the concentration of chlorophyll *a* in the treatment: 200  $\mu\text{g}/\text{L}$  of OC, remaining around 3:1 in the first 24 h, followed by an increase in chlorophyll *b* in the following periods, ending the 120 h with a ratio of 1:1. A similar behavior was observed in the 600  $\mu\text{g}/\text{L}$  OC treatment, with a significant increase in chlorophyll *a* at 24 h and an increase in chlorophyll *b* in the following hours (Figure 2b).

In the 400  $\mu\text{g}/\text{L}$  OC treatment, the chlorophyll *a/b* ratio was below 1 at all hours, and compared to the control, there was a proportional increase in the two chlorophylls (Figure 2a,b). This treatment accumulated the most OC in the floating portions in relation to the submerged portions, which certainly influenced this difference in reaction in relation to the other treatments. Souza et al. [18] observed that the concentration accumulating more chromium was also the one that showed the greatest drop in chlorophyll production, which indicates that chlorophyll production is impaired because the enzymes responsible for its production are reduced.

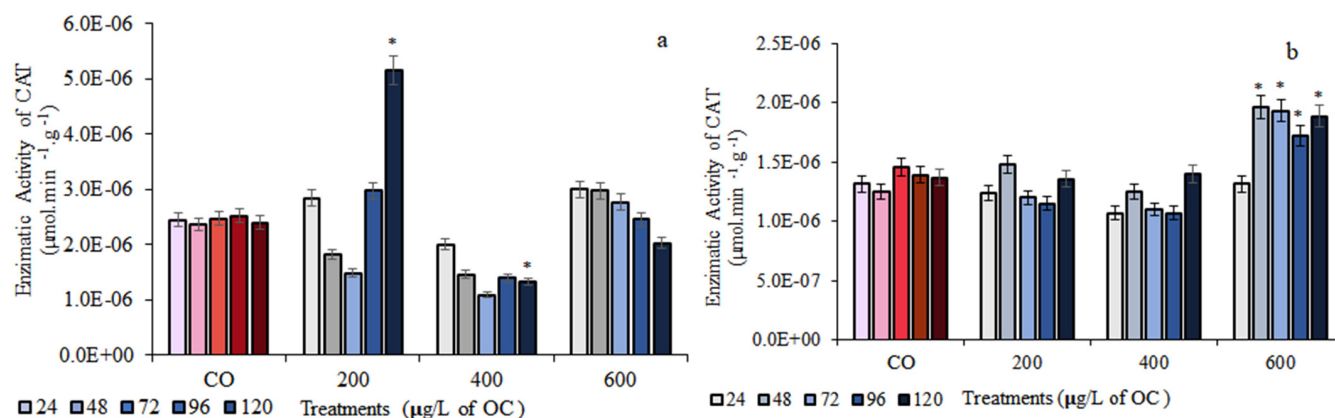
It was found that during the absorption of OC, metabolic changes occurred in the plant organism, reflected mainly in the increase in chlorophyll *a* in treatments 200 and 600 and in chlorophyll *b* in treatment 400. The plant’s reaction to treatment 400 indicates greater sensitivity of the plant to this OC concentration.

Changes in the composition of chlorophylls are expected as a response to oxygen-reactive substances. Reactive Oxygen Species (ROS),  $\text{H}_2\text{O}_2$ , being the most stable, is the most likely to occur and acts as a mobility signal [37]. Chloroplast ROS induce acclimatization responses through signaling to the nucleus so that adjustments are made in gene expression and plant-wide systemic signaling pathways [38]. Parenchymal plastids of the vascular system and bundle sheath signal and regulate the development of the mesophyll plastid [39].

The increase in chlorophyll *b* concentration is related to the plant’s ability to adjust to the stress caused by the accumulation of OC in the floating parts (Figure 1). Phytochrome B acts on the auxin pathways that transmit signals perceived by the apical leaves to increase the speed of induction of photosynthesis in the distal leaves [40].

The evaluation of the enzymatic system showed different activity between the submerged and floating portions of the plants. In the submerged portions, CAT was inhibited in the first 48 h in the treatments of 200 and 400  $\mu\text{g}/\text{L}$  of OC (Figure 3a), being below  $2.0\text{E}^{-6}$   $\mu\text{mol}/\text{min}/\mu\text{g}$  of protein, compared to the control that is above  $2.5\text{E}^{-6}$   $\mu\text{mol}/\text{min}/\mu\text{g}$  of protein. At the end of the experiment, there is a reaction of this enzyme with a great increase in activity in the treatment of 200  $\mu\text{g}/\text{L}$  ( $H = 10.24$  and  $p < 0.01$ ) above  $5.0\text{E}^{-6}$   $\mu\text{mol}/\text{min}/\mu\text{g}$  of protein. In the floating portions, the CAT reaction was indifferent

to the concentration and time of the experiment, with no statistically significant differences between treatments and times ( $H = 9.8$  and  $p = 0.02$ ), except after 48 h in the 600  $\mu\text{g/L}$  OC treatment, there was an increase in activity (Figure 3b), significantly different from the control, exceeding  $2.0\text{E}^{-6}$   $\mu\text{mol}/\text{min}/\mu\text{g}$  of protein. The small change in CAT activity in the submerged portions shows that the accumulation of  $\text{H}_2\text{O}_2$  in these plant tissues was small, but in the floating portion of treatment 600, there was an accumulation of  $\text{H}_2\text{O}_2$ , which led to intense activity of this enzyme.



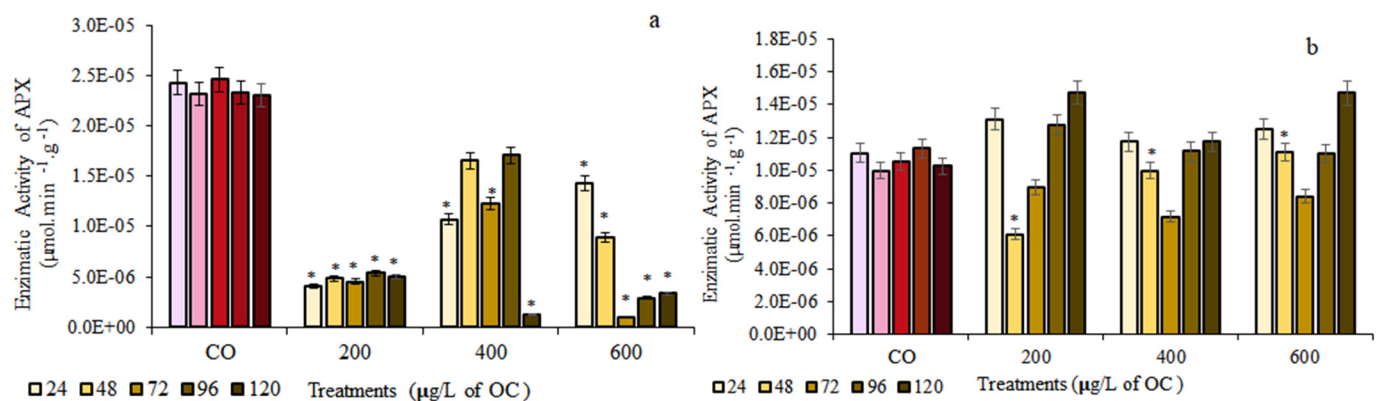
**Figure 3.** Activity of enzyme catalase (CAT), (a) submerged and (b) floating portions of the *S. biloba* at concentrations of 200, 400, and 600  $\mu\text{g/L}$  Octocrylene, for 120 h (5 days). \* Indicate statistically significant differences in concentrations and control, according to Kruskal–Wallis followed by Dunn’s test ( $p \leq 0.05$ ). CO: control (highlighted in red).

The inhibition of CAT with the treatment period indicates that saturation of the enzymatic activity occurred, and in the 200 and 400  $\mu\text{g/L}$  solutions of OC, the cells of the submerged portion of *S. biloba* were exposed to the deleterious effects of  $\text{H}_2\text{O}_2$ . The floating portion, however, kept activity levels close to control with little inhibition in the intermediate periods of the experiment. This lower alteration of CAT in the floating portions can be explained by its association with peroxisomes, which are found in greater quantities in the photosynthetic portions of plants.

CAT is an enzyme whose activity increases when the concentration of  $\text{H}_2\text{O}_2$  increases and is an enzymatic protector against peroxidation reactions [41]. Because it acts without the need for reducing agents [42], it has action and rapid response capacity, being one of the first in the metabolic system to come into action. The inhibition of the activity of this enzyme leaves the cell susceptible to the action of hydrogen peroxide, which can lead to serious metabolic consequences, such as the formation of hydroxyls and superoxides, complex substances that easily bind to proteins, enzymes, and nucleic acids, causing serious damage to cellular metabolism. Santo et al. [5] observed increased CAT activity after 48 h of contact at concentrations starting at 10  $\mu\text{g/L}$  of OC.

APX in the submerged portions was inhibited at all concentrations from the first hours of the experiment (Figure 4a) and remained low throughout the treatment, with treatments 200 and 600  $\mu\text{g/L}$  of OC standing out in relation to the control ( $H = 12.44$  and  $p < 0.008$ ), remaining below  $5.0\text{E}^{-6}$   $\mu\text{mol}/\text{min}/\mu\text{g}$  of protein in these treatments after 72 h. The enzymatic behavior in the floating portion was initially inhibited after 48 h of the experiment and returned to enzymatic activity from 96 h onwards at all concentrations (Figure 4b), but there was no significant difference in relation to the control only between treatment times ( $H = 14.18$  and  $p = 0.002$ ). APX activity drops to less than  $6.0\text{E}^{-6}$   $\mu\text{mol}/\text{min}/\mu\text{g}$  of protein in the treatment of 200  $\mu\text{g/L}$  of OC. Unlike CAT, APX needs a cofactor and uses ascorbate as a precursor to  $\text{H}_2\text{O}_2$  degradation; this gives it the ability to act at low concentrations of peroxide but is inhibited when  $\text{H}_2\text{O}_2$  levels increase [42].

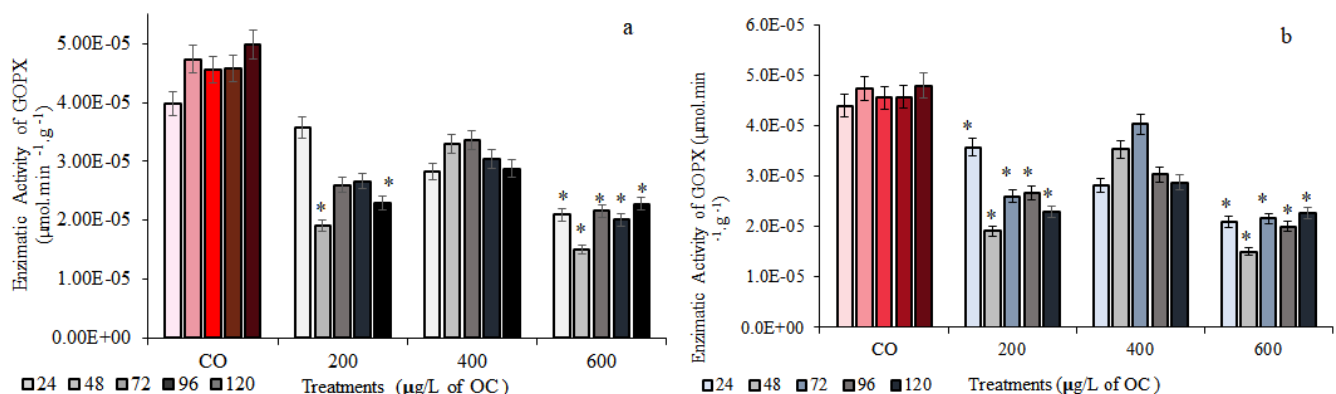




**Figure 4.** Activity of enzyme ascorbate peroxidase (APX) (a) submerged and (b) floating portions of the *S. biloba* at concentrations of 200, 400, and 600 µg/L Octocrylene for 120 h (5 days). \* Indicate statistically significant differences in concentrations and control, according to Kruskal-Wallis followed by Dunn's test ( $p \leq 0.05$ ). CO: control (highlighted in red).

The inhibition of APX in the submerged portions indicates the accumulation of  $H_2O_2$  as an immediate response to the OC, and the response of the floating portions compared to the transport of the OC by the plant tissues indicates that after 72 h, there is an attempt to compensate for the harmful effects on the floating portions by the increase in the concentration of chlorophyll *a* in all treatments at the end of the first 24 h. The APX enzyme is activated to preserve cell cycle function and maintain cell proliferation [43]. Nascimento et al. [3], also observed an effect of increased CAT activity and APX inhibition in response to OC effects. Santo et al. [5], observed the effect of OC on APX activity after 48 h of exposure to a 1000 µg/L concentration of OC.

GPOX was inhibited at all OC concentrations in both submerged and floating portions (Figure 5), with reaction peaks between 48 and 72 h in both portions at concentrations of 200 and 400 µg/L of OC, but without the ability to approach the activity presented by the control plants. In the submerged portions, the activity of GPOX is on average  $5.0E^{-8}$  µmol/min/µg of protein, much lower than in the aerial portions, around  $4.0E^{-5}$  µmol/min/µg of protein. GPOX is a cell membrane protection enzyme, and its inhibition has left the cell exposed to lipid peroxidation. The  $H_2O_2$  radical is associated with reduced cell membrane permeability and induces lipid peroxidation, leading to increased ROS accumulation [44].



**Figure 5.** Activity of enzyme guaiacol peroxidase (GOPX) (a) submerged and (b) floating portions of the *S. biloba* at concentrations of 200, 400, and 600 µg/L Octocrylene for 120 h (5 days). \* Indicate statistically significant differences in concentrations and control, according to Kruskal-Wallis followed by Dunn's test ( $p \leq 0.05$ ). CO: control (highlighted in red).

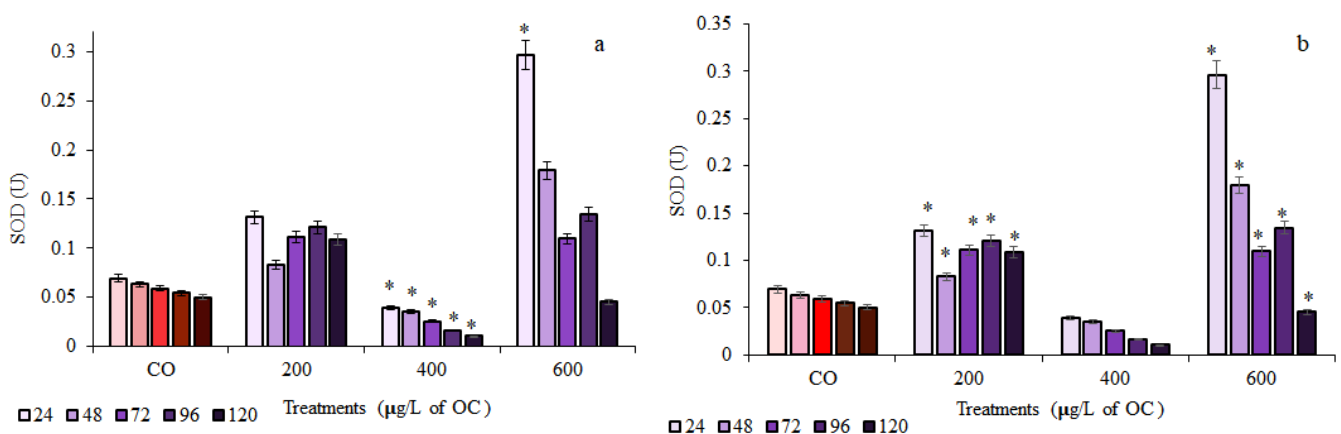
In the treatments of 200 and 600 µg/L of OC, the enzymatic activity decreased from  $2.0E^{-8}$  µmol/min/µg of protein in the submerged portion in 48 h of the exper-

iment and showed recovery in the following times ( $H = 15.5$   $p < 0.001$ ), not reaching  $3.0E^{-8}$   $\mu\text{mol}/\text{min}/\mu\text{g}$  of protein (Figure 5a). In the aerial portions, the behavior of the enzyme was very similar after 48 h of experiment with variations below  $2.0E^{-5}$   $\mu\text{mol}/\text{min}/\mu\text{g}$  of protein in the 200 and 600  $\mu\text{g}/\text{L}$  treatments of OC and recovery in the following times ( $H = 15.7$ ,  $p < 0.001$ ), but not exceeding  $3.0E^{-5}$   $\mu\text{mol}/\text{min}/\mu\text{g}$  of protein (Figure 5b).

The attempted reaction of GPOX indicates that the enzyme system is activated for the production of a protective enzyme, but with little success, as it does not present continuity in the following times. This is an indication that OC acts on plant nucleic acids and that hydroxyl radicals may have been formed as a result of its action. The hydroxyl radical causes damage to DNA, RNA, proteins, lipids, and cell membranes of the nucleus and mitochondria. In DNA, it attacks both nitrogenous bases and deoxyribose. Attacks on the amino acids that make up proteins can generate damage such as bond cleavages with or without the generation of fragments and cross-links, which can result in loss of enzymatic activity, difficulties in active transport through cell membranes, cytolysis, and cell death [45].

The activity of this enzyme in the 400  $\mu\text{g}/\text{L}$  OC treatment, although it was inhibited, is not statistically significant, and the response capacity was faster than in the other treatments, always above  $3.0E^{-8}$   $\mu\text{mol}/\text{min}/\mu\text{g}$  of protein in the submerged portion and  $3.0E^{-5}$   $\mu\text{mol}/\text{min}/\mu\text{g}$  of protein in the aerial portion (Figure 5b). This enzymatic response may explain the formation of chlorophyll *b* observed in this treatment (Figure 2a), because although these plants had the lowest values of chlorophyll *a/b* ratio, they were the ones that presented the most stable ratio throughout the treatment, due to the synthesis of chlorophyll *b*, which increased from 1237  $\mu\text{g}/\text{g}$  at 24 h of treatment to 2378  $\mu\text{g}/\text{g}$  at 48 h of treatment. There is a clear physiological response in the plant to adjust to the stressful situation and maintain its metabolism.

SOD is among the main defenders against  $\text{H}_2\text{O}_2$ . However, when it is highly active, it can generate superoxide, which is harmful to cellular metabolism. At 200  $\mu\text{g}/\text{L}$  of OC, the reaction to OC is immediate, with significant enzymatic activation from the first 24 h in the submerged portions (Figure 6a), going from 0.05 U to above 0.35 U. As the hours go by, there is a reduction in SOD activity (0.15 U after 120 h), but it remains well above control ( $H = 15.5$   $p < 0.001$ ).



**Figure 6.** Activity of enzyme superoxido dimutase (SOD) (a) submerged and (b) floating portions of the *S. biloba* at concentrations of 200, 400, and 600  $\mu\text{g}/\text{L}$  Octocrylene for 120 h (5 days). \* Indicate statistically significant differences in concentrations and control, according to Kruskal–Wallis followed by Dunn’s test ( $p \leq 0.05$ ). CO: control (highlighted in red).

In the floating portion, the treatments of 200 and 600  $\mu\text{g}/\text{L}$  of OC stand out significantly for the activation of SOD ( $H = 14.1$   $p < 0.002$ ), especially in the treatment of 600  $\mu\text{g}/\text{L}$  of OC, which exceeded 0.3U in the first 24 h and reacted with a reduction in the following hours (Figure 6b). SOD in the 400  $\mu\text{g}/\text{L}$  OC treatment shows inhibition behavior, both in the submerged and floating portions, reaching almost zero (Figure 6). The SOD response,

especially in the aerial portions, indicates that this enzyme acted to protect the plant organism and combat lipid peroxidation.

The inhibition of enzymatic activity is associated with the formation of superoxide; the formation of this radical is associated with a major stress condition, so it is important to evaluate the response to internal and external stimuli to provide tolerance to stress. ROS enzymes are sensitive to abiotic stress conditions, serving as stress signalers [46].

The behavior of *S. biloba* demonstrates its ability to absorb and translocate OC at all concentrations tested from root to leaf and to maintain metabolism in reasonable survival conditions, resuming photosynthesis at acceptable survival levels, but further tests will be needed to evaluate the long-term effects on plant metabolism, how long they could remain in the environment, and their level of efficiency over time. However, to indicate whether the accumulation is sufficient to demonstrate the phytotreatment potential of the medium, phytotoxic tests are very important and have corroborated its treatment capacity.

Regarding the cytotoxicity and genotoxicity tests in *A. cepa* to verify the efficiency of phytoextraction in Table 1, at time 0 h, it was verified that OC, at concentrations of 200 and 600 µg/L, caused damage to interphase, either by inhibition of DNA duplication or damage to protein synthesis, or both. This condition caused a significant reduction in cell division in the root meristems ( $17.9 \pm 0.9$  and  $19.4 \pm 0.7$ , respectively) when compared to the control with distilled water. This result corroborates Santo et al. [5], who evaluated this sunscreen at 100 and 1000 µg/L and found significant negative impacts on the cell division of *A. cepa* roots.

**Table 1.** Mitotic indices of root meristems from *A. cepa* bulbs exposed to Octocrylene for 120 h at three concentrations before (0 h) and after phytotreatment at different exposure times (24, 72, and 120 h).

Octocrileno (µg/L)	Exposure Time/Mitotic Index (%)			
	0 h	24 h	72 h	120 h
200	$17.9 \pm 0.9$ *	$21.2 \pm 0.7$ *	$43.2 \pm 0.6$	$45.0 \pm 0.7$
600	$19.4 \pm 0.7$ *	$18.0 \pm 0.9$ *	$35.1 \pm 0.9$	$41.0 \pm 0.9$
CO	$39.4 \pm 0.7$ *			

\* Indicate statistically significant differences between concentrations and controls (CO), according to Kruskal–Wallis, followed by Dunn’s test ( $p \leq 0.05$ ).

In Table 1, at 24 h of phytotreatment, the MI remained like that obtained for time 0 h. However, at 48 and 96 h of phytotreatment, cell division at the root tips for concentrations of 200 and 600 µg/L increased significantly, which presented higher MI than the control with distilled water ( $43.2 \pm 0.6$  and  $41.0 \pm 0.9$ ), proving to be non-cytotoxic.

In Table 2, at 0 h exposure time and at 24 h of phytotreatment, the concentrations of 200 and 600 µg/L were highly genotoxic, corroborating the inhibition of cell division observed for both concentrations in the same exposure time (Table 1). At 48 and 96 h of exposure, it was found that the solution in which the roots were found was no longer genotoxic. Based on Tables 1 and 2, it can be inferred that the phytoextraction of OC by *S. biloba* was effective at 48 and 96 h, with no significant differences between the two exposure times.

This demonstrates that *S. biloba*, after 72 h of contact with the contaminated medium, not only absorbed significant amounts of the contaminant but was also able to reduce it from the medium to less toxic levels, making the mitotic alteration effects equal to those of uncontaminated waters.

The germ test with terrestrial species showed that the species showed similar behavior at concentrations of 200 and 600 µg/L of OC. *A. fatua* germinates only after 120 h of treatment (Table 3); this is due to the characteristics of the species but with great differences in relation to the control. The effect of oxidative stress from OC on spontaneous plants and cultivars has been proven by Nascimento et al. [3], with inhibition of germination and elongation of roots.

**Table 2.** Indices of cellular alterations of root meristems from *A. cepa* bulbs exposed to 120 h of Octocrylene in two concentrations before (0 h) and after phytotreatment at different exposure times (0, 24, 72, and 120 h).

Octocrylene Conc (µg/L)		Number and Types of Cellular Changes				
Experiment Intervals		ET	Binucleated Cells	Metaphase Derangements	Anaphase Derangements	CAI ± SD (%)
200	0 h	19	n.d.	22	11	5.2 ± 0.8
	24 h	17	03	32	13	6.5 ± 0.7
	72 h	03	n.d.	n.d.	01	0.4 ± 0.5 *
	120 h	02	n.d.	n.d.	n.d.	0.2 ± 0.5 *
600	0 h	24	n.d.	31	18	7.3 ± 0.5
	24 h	21	14	29	18	8.2 ± 0.7
	72 h	02	n.d.	n.d.	0.4	0.6 ± 0.7 *
	120 h	n.d.	n.d.	n.d.	03	0.3 ± 0.8 *
CO		03	n.d.	n.d.	n.d.	0.3 ± 0.7 *

h: hours, Conc: concentration, ET: Exposure Time, CAI: Cell Alteration Index, SD: Standard Deviation. CO: control  
 \* Indicate statistically significant differences in concentrations and exposure time (0 h), according to Kruskal–Wallis, followed by Dunn’s test ( $p \leq 0.05$ ).

**Table 3.** Phytotoxicity of octocrylene, at concentrations of 200, 400, and 600 µg/L for *A. fatua* and *L. sativa* based on Relative Growth Index (RGI) and germination index (IG).

	Control			<i>A. fatua</i>			<i>L. sativa</i>	
	200	400	600	200	400	600	200	600
RGI								
24	1	1	1	0.14	0.13	0.05	0.30	0.30
72	1	1	1	0.32	0.34	0.23	0.48	0.48
120	1	1	1	0.52	0.51	0.33	0.97	0.97
IG								
24	100	100	100	14.28	13.74	2.81	30.24	30.24
72	100	100	100	32.33	34.35	23.30	48.10	48.10
120	100	100	100	52.63	51.14	33.83	97.59	97.59

Thus, when we verified that the cultivated and spontaneous plants presented germination above 50% *A. fatua* (barley/oats), reaching 97% *L. sativa*, the ability to extract OC from the medium by *S. biloba* was efficient, ensuring the quality of the water for use in cultivated species.

#### 4. Conclusions

Thus, evaluating the ability of *S. biloba* to absorb, transport, and accumulate OC in its submerged and aerial tissues, we can conclude that this species has OC phytoextractor capacity from small concentrations up to 600 µg/L. The physiological changes presented by the species show its ability to defend the organism, along with adjustments in photosynthetic production due to the alteration in the production of chlorophyll *b*. Ecotoxicity tests showed that after 72 h of treatment, the medium became less cytotoxic and genotoxic with the end of mitotic changes in *A. cepa* and with the reestablishment of the germination capacity of cultivated and spontaneous terrestrial species.

**Author Contributions:** All authors contributed to the study’s conception and design. Material preparation, data collection, and analysis were performed by M.A.S.M., G.C.S.G.N., O.V.J., A.P.P. and D.C.S. The first draft of the manuscript was written by D.C.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Conselho Nacional de Pesquisa (CNPQ) for the scientific initiation scholarship.

**Institutional Review Board Statement:** Project registered at SIsGen—National System for the Management of Genetic Heritage and Associated Traditional Knowledge, under the number: A4D42AC.

**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:** To Conselho Nacional de Pesquisa (CNPQ) for the scientific initiation scholarship to academic Matheus Moura.

**Conflicts of Interest:** The authors declare, there is no conflicts of interest in the research developed.

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