Phyton-
International Journal of
Experimental Botany

Tech Science Press

DOI: 10.32604/phyton.2022.020566

ARTICLE

Microalgae Improve the Photosynthetic Performance of Rice Seedlings (*Oryza sativa* L.) under Physiological Conditions and Cadmium Stress

Ekaterina Yotsova[1], Martin Stefanov[1], Georgi Rashkov[1], Margarita Kouzmanova[2], Anelia Dobrikova[1] and Emilia Apostolova[1,*]

[1]Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria

[2]Department of Biophysics and Radiobiology, Biological Faculty, Sofia University, Sofia, Bulgaria

[*]Corresponding Author: Emilia Apostolova. Email: emya@bio21.bas.bg

Received: 30 November 2021 Accepted: 19 January 2022

ABSTRACT

The aim of this study was to assess the impact of the microalgae *Chlorella vulgaris* on the rice seedlings at physiological conditions and under cadmium (Cd) stress. We examined the effects of *C. vulgaris* in the nutrient solution on rice seedlings grown hydroponically in the presence and the absence of 150 μM $CdCl_2$, using the low (77 K) temperature and pulse amplitude modulated (PAM) chlorophyll fluorescence, P700 photooxidation measurements, photochemical activities of both photosystems, kinetic parameters of oxygen evolution, oxidative stress markers (MDA, H_2O_2 and proline), pigment content, growth parameters and Cd accumulation. Data revealed that the application *C. vulgaris* not only stimulates growth and improves the functions of photosynthetic apparatus under physiological conditions, but also reduces the toxic effect of Cd on rice seedlings. Furthermore, the presence of the green microalgae in the nutrient solution of the rice seedlings during Cd exposure, significantly improved the growth, photochemical activities of both photosystems, the kinetic parameters of the oxygen-evolving reactions, pigment content and decreased lipid peroxidation, H_2O_2 and proline content. Data showed that the alleviation of Cd-induced effects in rice seedlings is a result of the Cd sorption by microalgae, as well as the reduced Cd accumulation in the roots and its translocation from the roots to the shoots.

KEYWORDS

Chlorella vulgaris; growth parameters; low temperature chlorophyll fluorescence; PAM chlorophyll fluorescence; photosynthesis; rice; stress markers

1 Introduction

Cadmium (Cd) is one of the most toxic heavy metals and even at trace amounts has harmful effects on the plant's development. It has been also found that Cd stress leads to an increase in the production of reactive oxygen species (ROS) in crop plants [1–3], which causes oxidative damage and thus inhibits the growth and the photosynthetic activity of plants [4–8]. Cadmium is a mobile element, easily absorbed by the roots, and transported to the shoots, that negatively affects plant growth [6]. It is well known that the harmful effects of Cd on the function of photosynthetic apparatus are a result of an influence on the chlorophyll metabolism, chloroplast ultrastructure, and on the activity of both photosystems [4,6,9,10]. It has been found that Cd reduces the chlorophyll content, alters the organization of the pigment-protein complexes of the thylakoid

membranes, which leads to the reduced quantum efficiency of photosystem I (PSI) and photosystem II (PSII) as well as the net photosynthetic rate [11]. Cadmium-induced changes on both the donor and the acceptor sides of PSII complex lead to an increase in the amount of inactive PSII centers [6,12]. The inhibition of the PSII activity is due to degradation of D1 protein of PSII complex, PsbO proteins of the oxygen-evolving complex (OEC) and damage the light-harvesting complex of PSII [13,14]. It has been proposed that the Cd competitive binding to the essential Ca^{2+} cofactor in the Mn_4Ca cluster of OEC also leads to inhibition of the oxygen evolution [15]. At the same time, the inhibitory effects on PSI activity were smaller in comparison to PSII [13].

Therefore, the study of various signaling molecules and microorganisms that alleviate Cd-induced stress is an important step for environmental ecology, including better protection of crop plants and their production from the adverse effects of heavy metals.

In recent years the interest in the application of cyanobacteria and green microalgae in ecological and integrated crop production has grown, especially [16–18]. Micro- and macroalgae have mechanisms that allow them to remove free metal ions from the aquatic environment, and thus detoxifying and purifying the water [19,20]. Microalgae are found in abundance in the environment. They live in marine and freshwater basins and have a photosynthetic apparatus like higher plants, accounting for 32% of the global photosynthesis [21]. As a result, the use of macro- and microalgae for phycoremediation (removal of contaminants) has grown in popularity in recent years due to several benefits including abundant availability, inexpensive, excellent metal removal efficiency and eco-friendly nature [18,22,23].

Microalgae cells can absorb toxic heavy metals from the environment, leading to higher concentrations of these metals in their cells than in the surrounding water [19,21,23,24]. The rich spectrum of mechanisms that allow microalgae to survive and thrive in the presence of heavy metals and at the same time to accumulate these metals in their cells, makes them suitable for practical application as a means of bioremediation (phycoremediation) [18,22,25]. It has been proposed that microalgae remove heavy metals directly from contaminated water in two main ways: the first is the metabolism-dependent uptake of metals by cells at low concentrations of contaminants, and the second is by biosorption, which is an inactive adsorption process [20,26,27]. Furthermore, the mechanisms by which microalgae remove heavy metals from the aquatic environment are: extracellular accumulation, which is more active in living microorganisms; cell surface sorption or complexation (active accumulation of heavy metals by covalent bonding from living and dead microorganisms); and intracellular accumulation, which can only take place by living microorganisms [18–20,23,28,29]. Microalgae can also synthesize peptides capable of binding heavy metals [30], which helps maintain an acceptable concentration of metal ions in the cytoplasm, thus preventing or neutralizing the potential toxic effect of heavy metals on cells [31]. *Chlorella* cells can contain up to 70% of protein (in dry weight), making the biomass very valuable to the food industry [20].

The affinity of microalgae to polyvalent metals makes them suitable for their potential application in the removal of metal ions from the aqueous medium, as particularly suitable for this purpose are the algae *Chlorella* and *Scenedesmus* [32]. Khan et al. [33] found that the unicellular alga *Chlorella vulgaris* can effectively remove Cd ions. Therefore, *C. vulgaris* is one of the most commonly reported species for heavy metal removal [20].

In recent years, some studies were focused on the influence of green microalgae on higher plants. It has been shown that microalgae stimulated germination and plant seedling growth, producing growth hormones, such as auxins, cytokinins, jasmonic acid, etc. [34,35]. The presence of *C. vulgaris* in the cultivation medium of *Lactuca sativa* promoted its growth and significantly increased fresh and dry weight of seedling, as well as pigment content [36]. Similar effects of this microalgae have been shown on sugar beet and tomato plants [37–40]. Some authors have suggested that the stimulation of pigment biosynthesis and the increased pigment content may improve photosynthetic activity [40], but there is no direct evidence. It has also

been shown that microalgae alleviate the oxidative damage in plants during drought stress and high salinity improves antioxidant defense system [41,42].

Despite numerous studies on the role of microalgae in the remediation of heavy metals, to our knowledge there is no data in the literature on the influence of microalgae on the photosynthetic functions of higher plants grown with or without heavy metals. We hypothesize that microalgae may mitigate the adverse effects of Cd action on rice plants. To test this hypothesis, we investigated the effect of the application of *C. vulgaris* cells in the nutrient medium of rice seedlings under physiological conditions and Cd stress by following the changes in oxidative stress markers, growth parameters, pigment content and photosynthetic functions of rice. In this study, the effects of *C. vulgaris* on the activity of the photosynthetic apparatus under physiological conditions and Cd stress were shown for the first time. The obtained data in the current study will contribute to the elucidation of mechanisms of plant tolerance, as well as the possibilities for exogenous application of *C. vulgaris* to reduce the harmful effects of heavy metals.

2 Materials and Methods

2.1 Plant Material and Cultivation of Green Algae

Rice (*Oryza sativa* L. Galileo) was chosen as plant material for this study. Rice seedlings were grown hydroponically under controlled conditions with a light intensity of 150–180 μmol photons m^{-2} s^{-1} and 12-h photoperiod at 28°C/20°C on plastic containers with half-strength Hoagland nutrient solution with modification: 2.5 mM KNO_3, 2.5 mM Ca $(NO_3)_2$, 1 mM $MgSO_4$, 0.5 mM NH_4NO_3, 23 μM H_3BO_3, 4.5 μM $MnCl_2$, 0.4 μM $ZnSO_4$, 0.2 μM $CuSO_4$, 0.25 μM Na_2MoO_4, 20 μM Fe-EDTA (pH 6.0) for 14 days. The nutrient solution was replaced every 4 days with a new one. Details for the cultivation of the plants are given in [3]. After germination, the seedlings were divided into four groups: (i) control group (grown only on in nutrient solution), (ii) grown on nutrient solution with *C. vulgaris*, (iii) grown on nutrient solution with 150 μM $CdCl_2$ and (iv) grown on nutrient solution with *C. vulgaris* and 150 μM $CdCl_2$. Our preliminary studies have found that the presence of *C. vulgaris* in the nutrient solution of rice seedlings with optical density OD_{760} = 1.2 has an optimal effect on the growth and the functional activity of the photosynthetic apparatus of plants.

The analyses were performed on seedlings, treated with *C. vulgaris* and/or $CdCl_2$ for 14 days. The measurements were made on fully expanded leaves and isolated thylakoid membranes. Thylakoid membranes were isolated from the rice leaves as described previously [43].

The *C. vulgaris* cells/culture was kindly provided by Institute of Plant Physiology and Genetics, Sofia, Bulgaria. Details for the cultivation of the microalgae are given in Rashkov et al. [44]. Algal suspensions in the exponential phase of growth were used in all experiments.

2.2 Pigment Analysis and Growth Parameters

Pigments were extracted from the rice leaves by grinding with ice cold 80% acetone. The amounts of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids (Car) were determined spectrophotometrically according the equations given in [45]. The measurements were made using Specord 210 Plus (Edition 2010, Analytik Jena AG, Germany). The pigment content in the leaves was calculated per gram fresh weight (g FW).

Growth parameters were determined by measuring the roots and shoot lengths at the end of the treatment with *C. vulgaris* and/or 150 μM $CdCl_2$.

2.3 Determination of Oxidative Stress Markers

The oxidative stress was assessed indirectly by measuring the levels of some oxidative stress markers in the leaves: malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and proline contents. The contents of H_2O_2,

MDA and proline in the rice leaves were determined as described previously in Yotsova et al. [3]. The data was given as nmol per g fresh weight (FW).

2.4 Pulse Amplitude Modulated (PAM) Chlorophyll Fluorescence

Pulse amplitude modulated (PAM) Chl fluorescence was measured on the fully expanded leaves using a fluorimeter (PAM 101-103, H. Walz, Effeltrich, Germany). The measurements were made as in Stefanov et al. [46]. Following parameters were determined: F_v/F_0-ratio of the photochemical to the nonphotochemical processes; Φ_{PSII}-effective quantum yield of the photochemical energy conversion of PSII, $(1 - q_P)$-amount of the closed PSII centers and the chlorophyll fluorescence ratio (R_{Fd}), which correlates with the photosynthetic rate [46,47].

2.5 P_{700} Redox-State Measurements

The measurements were made on the dark-adapted (for 15 min) leaves at room temperature using a PAM-fluorometer (Walz, Effeltrich, Germany) equipped with an ED-800T emitter-detector. The oxidation–reduction kinetics of P_{700} was determined by illumination of the dark-adapted detached leaves with fa-red light supplied by a photodiode (102-FR, Walz, Effeltrich, Germany). The changes in P_{700} oxidation (P_{700}^+) were determined by measuring the far-red light induced absorbance changes around 830 nm (ΔA) and the post-illumination dark reduction of P_{700}^+, which was fitted by two exponents with rate constants k_1 and k_2 [48].

2.6 Photochemical Activities of PSI and PSII

The PSII activity was assessed by the PSII-mediated electron transport ($H_2O_2 \rightarrow BQ$) and PSI activity by the PSI-mediated electron transport ($DCPIPH_2 \rightarrow MV$). The measurements were made polarographically using a Clark-type electrode (Model DW1, Hansatech, Instruments, Ltd., England) as described in [43].

2.7 Oxygen Evolution Measurements

Flash-induced oxygen yields were measured using a custom-built Joliot-type electrode [49]. The measurements were made as described in [3,43]. The effects of *C. vulgaris* and/or 150 μM $CdCl_2$ on the oxygen evolution were assessed using the following parameters: *A*–amplitude of the oxygen burst under continuous illumination; *Y*–maximum amplitude of the flash-induced oxygen yields; S_1–populations of PSII centers in the initial S_0–S_1 state distribution in the dark ($S_0 + S_1 = 100\%$); S_B–amount of the blocked PSII centers; α–the misses. The parameter S_B was obtained using an extended kinetic version of Kok's model (for details, see [44]).

2.8 Low-Temperature Fluorescence Measurements

The low-temperature (77 K) chlorophyll fluorescence emission spectra were used to assess the effect of Cd treatment on the energy distribution between the pigment-protein complexes of the photosynthetic apparatus. The measurements were made using a Jobin Yvon (JY3) spectrofluorometer equipped with a liquid-nitrogen device. The chlorophyll fluorescence was excited at 436 nm (for Chl *a*) and recorded from 650 to 780 nm with slit widths of 4 nm. The ratio F_{744}/F_{685} of the fluorescence maxima was used as a sensitive indicator of the energy redistribution between the two photosystems.

2.9 Determination of Cd Content

Determination of the Cd content was made by a specialized laboratory of University of Forestry, Sofia (EN ISO 6869). The Cd content was determined by the atomic absorption spectrometry technique (Perkin Elmer 5000, USA) at 228.8 nm. Details for the measurement and calculation of Cd content in plant tissues are shown in [43]. Translocation factor (TF = [Cd]shoot/[Cd]root) was calculated as in [50].

2.10 Statistical Analysis

The mean values (±SE) for all studied parameters were calculated from four independent treatments with three replicates per each treatment. Analysis of variance (ANOVA) and Tukey's post-hoc tests was used to determine the statistical differences between groups in the studied parameters. Values of $P < 0.05$ were considered as significantly different.

3 Results

3.1 Plant Growth and Pigment Content

The presence in the nutrient solution of *C. vulgaris* alone significantly increased the roots length (by 56%) compared to the control group of plants, while the shoots length increased by only 8% (Fig. 1). The experimental results also showed that the total chlorophyll content slightly increased (by 7%) (Fig. 2A), while there was not any statistically significant difference in the Carotenoids and Chl *a/b* ratio in comparison to the control group of plants (Figs. 2B, 2C). The Cd treatment alone resulted in a reduction in shoot and root lengths, as well as in the pigment content and an increase in the Chl *a/b* ratio. Data revealed that in rice plants grown in the combined presence of $CdCl_2$ and *C. vulgaris*, the Cd-induced changes in root and shoot length, and pigment content were smaller than that in the plants treated with Cd alone.

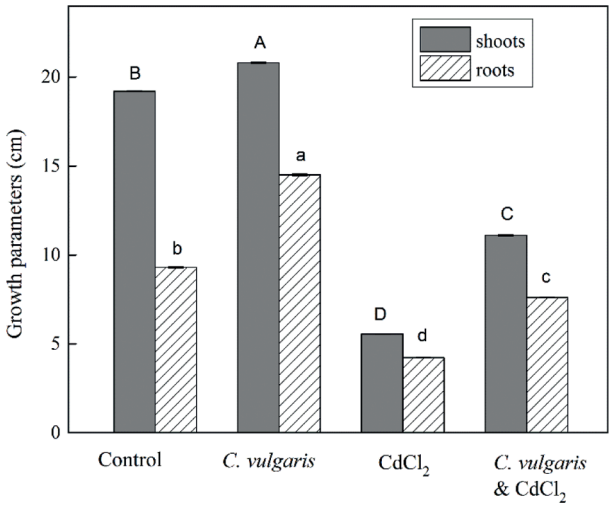

Figure 1: Effects of *C. vulgaris* and/or 150 µM $CdCl_2$ on the growth parameters (roots and shoots) of rice seedlings. Mean values (± SE) are calculated from four independent treatments with three replicates each. Different letters indicate significant differences at $P < 0.05$

3.2 Oxidative Stress Markers

The cultivation of rice plants in the presence of *C. vulgaris* alone in the nutrient solution caused a decrease in the leaf contents of H_2O_2 (by about 20%), MDA (by 42%) and proline (by 47%) in comparison with the control plants (Table 1). The Cd stress led to an increase in the content of H_2O_2 in the leaves by about 25%. The increase in H_2O_2 can lead to lipid peroxidation. The damage of the membrane lipids in rice plants under Cd stress was assessed by determination of the MDA content in the leaves. Experimental results showed a significant increase in MDA content (by 90%) in rice plants grown in the presence of $CdCl_2$. The Cd stress also caused a strong increase (nearly three times) in the amino acid proline in the leaves of rice plants compared to the control plants (Table 1).

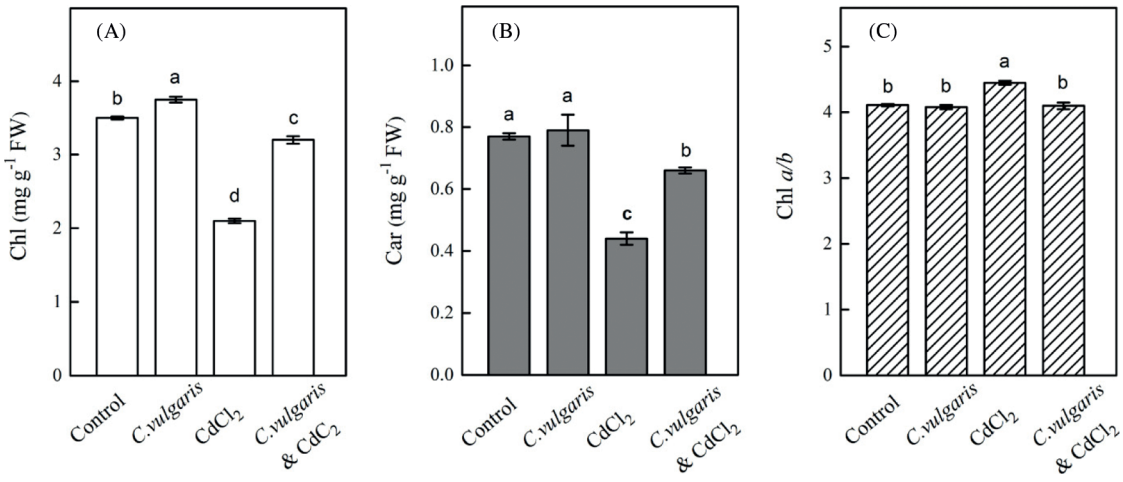

Figure 2: Effects of *C. vulgaris* and/or 150 μM $CdCl_2$ on the pigments content–total Chl (A), Car (B) and Chl *a*/*b* (C) in rice seedlings. Mean values (± SE) are calculated from four independent treatments with three replicates per each treatment. Different letters indicate significant differences at $P < 0.05$

Table 1: Effects of *C. vulgaris* on the MDA, H_2O_2 and proline contents in leaves of rice seedlings under physiological conditions or Cd stress

Parameter	Control	*C. vulgaris*	$CdCl_2$	*C. vulgaris* & $CdCl_2$
MDA (nmol g^{-1} FW)	102.1 ± 5.0^{c}	59.1 ± 1.9^{d}	202.0 ± 2.7^{a}	112.0 ± 2.6^{b}
H_2O_2 (nmol g^{-1} FW)	109.1 ± 3.6^{b}	86.8 ± 2.8^{c}	138.3 ± 3.8^{a}	102.3 ± 2.1^{b}
Proline (nmol g^{-1} FW)	210.2 ± 2.1^{c}	112.0 ± 3.4^{d}	633.8 ± 1.5^{a}	422.3 ± 1.5^{b}

Note: Mean values (±SE) are calculated from four independent treatments with three replicates per each treatment. Different letters indicate significant differences between the values in the same row ($P < 0.05$).

The observed increase in the levels of studied oxidative stress markers after the treatment with $CdCl_2$ was significantly alleviated by the presence of *C. vulgaris* in the nutrient solution of the rice plants, as the increase of MDA was only 15%, the proline levels were doubled, and the H_2O_2 content did not change compared to the control (Table 1).

3.3 PAM Chlorophyll Fluorescence Parameters

Analysis of the PAM chlorophyll fluorescence curves of leaves from rice plants grown in the presence of *C. vulgaris* showed an increase in the studied parameters (Fig. 3), the values of F_v/F_0 by 19%, Φ_{PSII} by 18%, R_{Fd} by 16% compared to the control group of plants.

Treatment with $CdCl_2$ alone resulted in a decrease of the chlorophyll fluorescence parameters Φ_{PSII}, F_v/F_0 and R_{Fd} (from 9% to 13%) and an increase of the close PSII centers ($1 - q_P$) (Fig. 3). Combined treatment of the rice plants with $CdCl_2$ and *C. vulgaris* did not show a statistically significant alteration in the studied parameters (F_v/F_0, Φ_{PSII}, R_{Fd} and $1 - q_P$) compared to the control values indicating a protection of *C. vulgaris* from Cd-induced effects on PSII photochemistry. The results clearly showed the protective role of green microalgae in conditions of Cd stress.

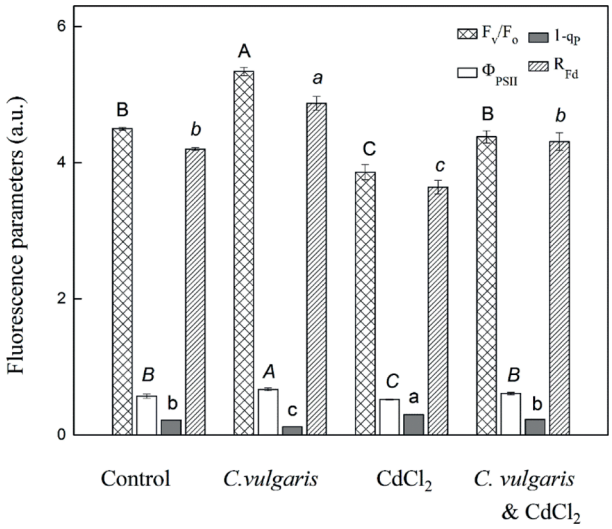

Figure 3: Effects of *C. vulgaris* and/or 150 μM $CdCl_2$ on PAM chlorophyll fluorescence parameters: Fv/F_0-ratio of the photochemical to the nonphotochemical processes; Φ_{PSII}-effective quantum yield of the photochemical energy conversion of PSII and $(1 - q_P)$-amount of the closed PSII centers and the chlorophyll fluorescence decrease ratio, R_{Fd}, which correlates with the photosynthetic rate. Mean values (± SE) are calculated from four independent treatments with three replicates per each treatment. Different letters indicate significant differences for the corresponding parameter at $P < 0.05$

3.4 Low-Temperature (77 K) Chlorophyll Fluorescence Measurements

Analysis of the 77 K chlorophyll fluorescence spectra showed that the presence of *C. vulgaris* in the nutrient solution of the rice plants did not lead to a change in the F_{744}/F_{685} ratio (Table 2), i.e., the redistribution of excitation energy between the two photosystems was not affected. The data also showed that the treatment with $CdCl_2$ led to an increase in this ratio, which is associated with an increase in the energy transfer from PSII to PSI, while the addition of *C. vulgaris* during Cd stress prevented this increase.

Table 2: Effects of *C. vulgaris* on the light-induced oxidation of P_{700} and the low-temperature chlorophyll fluorescence ratio F_{744}/F_{685} under physiological conditions and Cd stress

Parameter	Control	*C. vulgaris*	$CdCl_2$	*C. vulgaris* & $CdCl_2$
ΔA/A (%)	100 ± 1.09^a	101.5 ± 1.32^a	80.26 ± 1.18^c	90.6 ± 1.11^b
k_1 (s^{-1})	0.62 ± 0.08^b	0.67 ± 0.09^b	1.16 ± 0.02^a	0.70 ± 0.03^b
k_2 (s^{-1})	0.11 ± 0.05^b	0.12 ± 0.02^b	0.22 ± 0.03^a	0.12 ± 0.03^b
F_{744}/F_{685}	1.06 ± 0.05^b	1.14 ± 0.03^b	1.40 ± 0.06^a	1.17 ± 0.06^b

Note: ΔA/A–relative changes of far-red light induced oxidation of P_{700} ; k_1 and k_2 constants of fast and slow components of P_{700}^+ dark relaxation; Mean values (±SE) are calculated from four independent treatments with three replicates per each treatment. Different letters indicate significant differences between the values in the same row ($P < 0.05$).

3.5 Oxidation–Reduction Kinetics of P_{700}

For characterization of PSI photochemistry, we have measured the steady-state P_{700} photo-oxidation (P_{700}^+) by far-red light-induced absorbance changes around 830 nm (ΔA). The post illumination dark-reduction kinetics of P_{700}^+ were fitted by two decay exponents with constants k_1 (for the fast component)

and k_2 (for the slow component). Values of the relative amplitudes (ΔA/A) and the values for the constants (k_1 and k_2) from control and treated plants are shown in Table 2.

The presence of the green microalgae alone in the nutrient solution did not lead to changes in the ratio ΔA/A as well as the constants k_1 and k_2, which are similar to control plants (Table 2). Cadmium stress reduced the relative amount of P_{700}^{+} (the ΔA/A parameter decreased by about 20%), which indicates an influence on the photochemistry of PSI in plants treated with $CdCl_2$ alone. Cadmium treatment alone also increased the constant k_1 (by 45%) and k_2 (by 50%) compared to control plants. Combined treatment with $CdCl_2$ and *C. vulgaris*, alleviated the effects of Cd stress (values are close to those of the plants grown in the presence of *C. vulgaris* alone), indicating a protective effect of the green microalgae under conditions of heavy metal stress.

3.6 Photochemical Activity of PSII and PSI, and Oxygen-Evolution Parameters

The presence of green microalgae *C. vulgaris* in the nutrient solution led to stimulation of PSII-dependent electron transport by about 27%, while the activity of PSI did not change in comparison to the control plants (Table 3). In rice plants treated only with 150 μM $CdCl_2$, a significant inhibition of the PSII activity was observed (the electron transport $H_2O \rightarrow BQ$ was inhibited by 46%), while the PSI activity was much less influenced (electron transport $DCPIPH_2 \rightarrow MV$ was inhibited by 7%). The presence of *C. vulgaris* during Cd stress reduced the Cd-induced changes, as the inhibition of PSII was only 15%, while the photochemical activity of PSI was similar to that of control plants (Table 3).

Table 3: Effects of *C. vulgaris* on the photochemical activities of PSII and PSI, as well as on the kinetic parameters of oxygen evolution under physiological conditions and Cd stress

Parameter	Control	*C. vulgaris*	$CdCl_2$	*C. vulgaris* & $CdCl_2$
PSII activity	45.91 ± 1.10^{b}	58.34 ± 2.32^{a}	24.88 ± 1.83^{d}	38.80 ± 1.34^{c}
PSI activity	174.22 ± 2.77^{a}	175.31 ± 3.14^{a}	161.65 ± 1.52^{b}	178.91 ± 4.04^{a}
A (%)	100.00 ± 0.57^{b}	117.05 ± 3.12^{a}	61.40 ± 4.81^{d}	92.21 ± 5.14^{c}
Y (%)	100.00 ± 0.53^{b}	121.13 ± 4.95^{a}	40.22 ± 1.04^{d}	78.61 ± 2.30^{c}
S_1 (%)	73.82 ± 0.05^{c}	74.80 ± 0.08^{c}	32.99 ± 2.20^{a}	53.30 ± 2.51^{b}
S_B (a.u.)	1.39 ± 0.07^{b}	1.43 ± 0.15^{b}	1.90 ± 0.03^{a}	1.51 ± 0.14^{b}
α (%)	27.02 ± 1.01^{b}	27.10 ± 0.09^{b}	38.03 ± 1.80^{a}	28.2 ± 1.20^{b}

Note: *Y*-the flash-induced oxygen yields; *A*-the amplitude of oxygen evolution under continuous illumination; S_1 (%)–the PSII centers in S_1 state in the dark ($S_0 + S_1 = 100\%$); S_B–the amount of the blocked PSII centers; α–the misses. Mean values (±SE) are calculated from four independent treatments with three replicates per each treatment. Different letters indicate significant differences between values in the same row ($P < 0.05$).

The maximum amplitude of the flash-induced oxygen yields observed after the third flash (*Y*) and the amplitude of oxygen evolution under continuous illumination (*A*) were used to assess the influence of the green microalgae on the oxygen-evolving complex (OEC) in Cd-stressed rice plants (Table 3). The parameter *A* correlates with the number of all functionally active PSII reaction centers (i.e., fast and slow operating centers), while the parameter *Y* characterizes mainly the fast operating PSII centers situated in grana domains (see [51]). The cultivation of the rice plants in the presence of *C. vulgaris* alone in the nutrient solution led to an increase in flash-induced oxygen yields and the amplitude of oxygen evolution under continuous illumination (21% for *Y* and 17% for *A*, Table 3). Our results revealed that the treatment with 150 μM $CdCl_2$ strongly inhibited the oxygen evolution, as the values of the studied parameters (*Y* and *A*) significantly decreased compared to the control and the effects were more pronounced for the flash-induced oxygen yields (*Y* decreased by 52%) than for *A* (by 39%). The inhibitory effects of Cd ions

on the oxygen evolution were much less pronounced in rice plants grown in the presence of *C. vulgaris* and $CdCl_2$ (*Y* decreased by 21% and *A* by 8%) compared to the controls, but the values remained lower than those of control plants (Table 3).

The cultivation of rice plants with *C. vulgaris* in the nutrient solution did not change the initial dark distribution of the PSII centers in the S_0–S_1 states, the parameter S_B and the misses (α) in comparison to the control plants (Table 3). In rice plants exposed to 150 μM $CdCl_2$ we observed an enhance of the number of blocked PSII centers (S_B) by 43% and of the PSII centers in the S_0 state in the dark (i.e., strongly decrease of the S_1 state), as well of the misses (α) by 11% compared to the control plants. The observed changes are probably due to a modification in the Mn-cluster and/or damage to the OEC in the donor side of PSII. The presence of *C. vulgaris* in the nutrient solution during treatment with 150 μM $CdCl_2$ reduced the negative effects of Cd ions on OEC (the S_0–S_1 state distribution in the darkness). The values for misses (α) and the number of blocked PSII centers (S_B) were similar to those of untreated plants (Table 3).

3.7 Accumulation of Cadmium in Rice Plants

To determine whether alleviated inhibitory effects of the Cd treatment by *C. vulgaris* is due to the different accumulation of Cd in the roots and shoots of the rice plants, the Cd content in microalgae cells and plant's tissues was measured (Table 4). Data showed that the application of the green microalgae during Cd stress decreased the Cd content in both roots and shoots of the rice plants. To assess the ability of the rice plants to translocate Cd from the roots to the shoots we determined the translocation factor (TF), which represents the ratio of Cd content in plant shoots to that in the roots [50]. The TF value was less than 1 under both treatments, as the Cd transport from roots to shoots was more restricted in the presence of *C. vulgaris* (TF = 0.146) than after treatment with 150 μM $CdCl_2$ alone (TF = 0.240) (Table 4). This indicates that the presence of *C. vulgaris* in the nutrient solution limited the transport of Cd ions from roots to the shoots. On the other hand, the absorption of Cd ions from the roots is reduced by about 64% after the application of *C. vulgaris*, due to the absorption of Cd by the green microalgae cells, who accumulated about 3852 μg. g^{-1} DW of this metal during the treatment with 150 μM $CdCl_2$.

Table 4: Effect of *C. vulgaris* on the translocation factor (TF) and the Cd content in rice seedling under Cd stress

Parameter	$CdCl_2$	*C. vulgaris* & $CdCl_2$
Cd in roots (μg. g^{-1} DW)	3290 ± 69^a	1170 ± 79^b
Cd in shoots (μg. g^{-1} DW)	789 ± 88^a	171 ± 37^b
TF	0.240 ± 0.021^a	0.146 ± 0.020^b

Mean values (±SE) are calculated from four independent treatments with three replicates per each treatment. Different letters in the same column denote significant differences ($P < 0.05$).

4 Discussion

The current study shows the effects of *C. vulgaris* on rice seedlings under physiological conditions as well as under Cd stress. Stimulation of the growth parameters and an increase in the chlorophyll content have been found in plants grown in the presence of *C. vulgaris* in the nutrient solution, which is probably due to the bioactive growth compounds contained in this microalga [20,52,53]. In addition, microalgae also contain amino acids that are well known for their positive effects on plant growth and yield, stimulating the biosynthesis of chlorophylls and carotenoids, which leads to improved photochemical activity and an increase in the leaf mass [54]. Later studies have found a beneficial effect of this alga on sugar beet roots

and an influence on the regulation of genes involved in various biological pathways of primary and secondary metabolism [38]. It has also been shown that in tomato plants cultivated with *C. Vulgari* the dry and fresh weight increased [37,55], which could be related to the continuous photosynthesis of algae, which constantly supplies oxygen to the hydroponic nutrient solution [38]. Another possible reason involved in the biostimulating action of microalgae belonging to *Chlorophyta* spp. is the production and excretion of hormones (auxins and cytokinins), vitamins and biostimulants in the medium involved in growth regulation [20,34,56,57]. Our experimental results revealed that the presence of *C. vulgaris* in the nutrient solution of the rice plants resulted in stimulation of the photochemistry of PSII (increased Φ_{PSII}) and the rate of photosynthesis (parameter R_{Fd}), and decreased of the amount of the closed PSII centers ($1 - q_P$) (Fig. 3). In contrast to PSII, the activity of PSI was not affected by the addition of green microalgae to the nutrient solution (Tables 2 and 3). At the same time, there was a decrease in the content of H_2O_2 (by about 20%), MDA (by 42%) and proline (by 47%) in the leaves of rice seedlings grown in the presence of *C. vulgaris* in the nutrient solution compared to the control group of plants (Table 1).

To test whether microalgae can mitigate the adverse effects of Cd ions on the photosynthetic apparatus of rice seedlings, we investigated the effects of *C. vulgaris* on the photosynthetic function under Cd stress caused with 150 μM $CdCl_2$. Our results showed a strong decrease in the chlorophyll and carotenoid leaf content of Cd-treated rice plants (Fig. 2), which is most likely the result of the influence on the chlorophyll biosynthesis [4]. The reduction in pigment levels has been previously observed in wheat [43,58], maize [59,60] and soybeans [61]. The changes in chlorophyll content under Cd stress was accompanied by an increase in the Chl *a/b* ratio (Fig. 2). Based on previous studies, which suggested that the Chl *a/b* ratio correlates with the amount of the light-harvesting complex II (LHCII) and the degree of thylakoid staking [51,62,63], we suggest changes in the organization of thylakoid membranes, which could be a result from the increased lipid peroxidation in the membrane under Cd stress (Table 1). The inhibitory effect of Cd was also accompanied with a decrease in the growth parameters (Fig. 1). Alleviation of the effect of Cd stress on the pigment composition and growth parameters was found in the presence of *C. vulgaris* (Figs. 1 and 2). The protective effect of green microalgae is likely suppression of H_2O_2 accumulation and lipid peroxidation rate (Table 1). The exact mechanism is unknown, but microalgae limit Cd availability by sorption Cd ions [64] and also seem to support barriers preventing Cd translocation to the shoots. The cell surface is the focal point for the binding of metals to algae [65], and the sorption of heavy metals involves the exchange of metal ions with surface-bound cations or protons [66]. Ji et al. [67] also found that live algae have a higher ability to remove metals from inanimate microalgae biomass. The presence of *C. vulgaris* during the cultivation of peppers (*Capsicum annuum* L.) has been also found to lead to significantly reduced ROS production and lower lipid peroxidation [68]. In addition, it has been reported that the use of metal-resistant endophytic bacteria can also interact directly with heavy metals and thus reduce the accumulation of Cd in rice plants (*Oryza sativa L.*) improving plant growth and regulating their antioxidant system and endogenous hormones [69,70].

In addition, our data demonstrated that Cd-induced changes in the thylakoid membrane influenced energy distribution between chlorophyll-protein complexes (Table 2). Data revealed an increase in the F_{744}/F_{685} ratio, i.e., an increase in the energy transfer from PSII to PSI, which could be a result from Cd-induced changes of LHCII organization, reducing the amount of the trimers of this complex and eventually the efficiency of light energy utilization [71]. The experimental results showed that the presence of the *C. vulgaris* in nutrient medium prevent Cd-induced influence on the energy distribution between pigment-protein complexes (Table 2).

Cadmium ions have strong impact on the electron transfer reactions in the PSII complex, which leads to inhibition of oxygen evolution (Table 3) confirming previous observations [12,72,73]. In addition, it has been shown that Cd affects both the donor and acceptor side of the PSII, influencing the conformation of the Q_B binding side on the acceptor side of PSII and blocks electron transfer from Q_A to Q_B [74]. PAM chlorophyll

fluorescence measurements in our study showed inhibition of the effective quantum yield of PSII (Φ_{PSII}), an increase of the close PSII reaction centers ($1 - q_P$) and decrease of the photosynthesis rate (R_{Fd} parameter) (Fig. 3), which is a result from Cd-induced changes in the photosynthetic apparatus. It has also been suggested that Cd rapidly inhibits the donor side between OEC and the primary electron donor of P_{680} (Y_Z) [74]. In our previous investigations on rice plants [3], we have shown that Cd influences the kinetic parameters of oxygen-evolving reactions, as the effect was stronger on the PSIIα centers than PSIIβ centers. The Cd-induced increase in the number of inactive PSII reaction centres and inhibition of the OEC functions was also observed in wheat seedlings [12,43]. Our current results about oxygen evolution also showed inhibition of the oxygen evolution (*Y* and *A* amplitudes) and PSII-dependent electron transport ($H_2O_2 \rightarrow BQ$) (Table 3). Data also revealed that the presence of *C. vulgaris* in the nutrient solution reduces the toxic effects of Cd ions on the PSII photochemistry and oxygen evolution (Table 3). In addition, the experimental results showed a protective effect of *C. vulgaris* against Cd stress on oxygen-evolving parameters (flash yields *Y* and oxygen burst amplitude *A*), maintaining the amount of active PSII centers (i.e., S_B decreased) and the misses (α) with values close to the control (Table 3), which implies protection of OEC from damage or modifications.

Cadmium-induced alterations in the photosynthetic apparatus were less pronounced in PSI photochemistry than in PSII photochemistry (Table 3). The kinetics of the dark reduction of P_{700}^{+} give additional information about the influence of the Cd toxicity on the PSI complex. Data revealed some decrease of the ΔA/A ratio and an increase of the constants k_1 and k_2, which suggest changes in this complex (Table 2). These changes may be due to the destruction of the iron-sulfur centers and/or the antenna complex of PSI [4,12,72]. The effect of Cd treatment on both constants (k_1 and k_2) is probably due to changes occurring in both populations of PSI in the grana margin and in the stromal lamellae area. Our data revealed a protective effect of *C. vulgaris* on the PSI photochemistry under Cd stress conditions. The protective effect of *C. vulgaris* was not associated with a change in the constant k_1, corresponding with an influence on the cyclic electron transport around PSI (Table 2).

In a previous study, we have shown the protective mechanism of salicylic acid (SA) on rice under Cd stress [3] . The experiments in this study were conducted under the same conditions, which allows us to compare the effects of *C. vulgaris* with the known protective action of SA under Cd stress. The comparison showed that the effects of Cd on growth parameters, pigment composition and lipid peroxidation were much less pronounced in the presence of *C. vulgaris* in comparison to SA. Data also showed that the protective effects of green microalgae and SA [3] are almost the same on the photochemical energy conversion in PSII, the amount of the open PSII centers, the rate of the photosynthesis (R_{Fd} parameter) (Fig. 3), as well as the PSI photochemistry under Cd stress (Table 3). One of the protective mechanisms of the SA under Cd stress is the stimulation of PSI-dependent cyclic electron transport [3], which is not observed in the presence of green microalgae (Table 2). It should also be noted that the addition of *C. vulgaris* to the nutrient solution decreased the accumulation of Cd in the roots and shoots of the rice plants due to its sorption by the green microalgae cells as proposed previously [18–20,23], while the treatment with SA more severely restricted transport of Cd from the roots to the leaves, i.e., TF factor in the present of SA is lower (0.105 ± 0.018, unpublish data) than that after addition of *C. vulgaris* (0.146 ± 0.020, Table 4). A protective effect has been shown previously for the microalgae *Spirulina maxima* and *Chlorella ellipsoida*, which increased wheat tolerance to salinity by increasing antioxidant protection [42]. Stimulation of antioxidant protection in the presence of *C. vulgaris* under Cd stress cannot be excluded.

In summary, the experimental results in the current study showed that the application of *C. vulgaris* in the nutrient solutions stimulates plant's growth and increases the chlorophyll content, as well as improves the functional activity of the photosynthetic apparatus. The observed effects are a result of the biostimulating action of the microalgae, which synthesize and exclude in the medium hormones of growth regulation.

The application of green microalgae under Cd stress reduced its toxic effects in the rice seedlings by absorbing the ions of this heavy metal, as well as by reducing the Cd accumulation in roots and the Cd translocation from roots to shoots.

Author Contributions: Conceptualization, E.A.; methodology, E.Y., M.S., G.R., A.D. and E.A.; investigation, E.Y., M.S., G.R., and A.D.; writing—original draft preparation, E.A.; writing—review and editing, E.Y., M.S., A.D. and E.A.; All authors have read and agreed to the published version of the manuscript.

Acknowledgement: The authors thank the colleagues from the Laboratory "Experimental Algology" of the Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences for the provided *Chlorella* cells. The authors also express their gratitude to Daniela Stanoeva for the help in performing some of the biochemical measurements.

Funding Statement: This work was financially supported by the Project No. 137/12.05.2016 of the Program for Career Development of Young Scientists, Bulgarian Academy of Sciences.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

1. Vestena, S., Cambraia, J., Ribeiro, C., Oliveira, J. A., Oliva, M. A. (2011). Cadmium-induced oxidative stress and antioxidative enzyme response in water hyacinth and salvinia. *Brazilian Journal of Plant Physiology, 23(2),* 131–139. DOI 10.1590/S1677-04202011000200005.
2. Zhao, F. Y., Han, M. M., Zhang, S. Y., Wang, K., Zhang, C. R. et al. (2012). Hydrogen peroxide-mediated growth of the root system occurs via auxin signaling modification and variations in the expression of cell-cycle genes in rice seedlings exposed to cadmium stress. *Journal of Integrative Plant Biology, 54(12),* 991–1006. DOI 10.1111/j.1744-7909.2012.01170.x.
3. Yotsova, E. K., Dobrikova, A. G., Stefanov, M. A., Kouzmanova, M., Apostolova, E. L. (2018). Improvement of the rice photosynthetic apparatus defence under cadmium stress modulated by salicylic acid supply to roots. *Theoretical and Experimental Plant Physiology, 30(1),* 57–70. DOI 10.1007/s40626-018-0102-9.
4. Parmar, P., Kumari, N., Sharma, V. (2013). Structural and functional alterations in photosynthetic apparatus of plants under cadmium stress. *Botanical Studies, 54(1),* 45. DOI 10.1186/1999-3110-54-45.
5. Zhu, D., Ke, X., Wu, L., Li, Z., Christie, P. et al. (2016). Ecotoxicity of cadmium in a soil collembolan-predatory mite food chain: Can we use the 15N labeled litter addition method to assess soil functional change? *Environmental Pollution, 219,* 37–46. DOI 10.1016/j.envpol.2016.09.051.
6. Dobrikova, A. G., Apostolova, E. L. (2019). Damage and protection of the photosynthetic apparatus under cadmium stress. *Cadmium Toxicity and Tolerance in Plants,* pp. 275–298. Cambridge, MA, USA: Academic Press. DOI 10.1016/B978-0-12-814864-8.00011-5.
7. Lv, X., Fang, Y., Zhang, L., Zhang, W., Xu, L. et al. (2019). Effects of melatonin on growth, physiology and gene expression in rice seedlings under cadmium stress. *Phyton-International Journal of Experimental Botany, 88(2),* 91–100. DOI 10.32604/phyton.2019.06622.
8. Zulfiqar, U., Ayub, A., Hussain, S., Waraich, E. A., El-Esawi, M. A. et al. (2021). Cadmium toxicity in plants: Recent progress on morpho-physiological effects and remediation strategies. *Journal of Soil Science and Plant Nutrition*. DOI 10.1007/s42729-021-00645-3.
9. Djebali, W., Zarrouk, M., Brouquisse, R., El Kahoui, S., Limam, F. et al. (2005). Ultrastructure and lipid alterations induced by cadmium in tomato (*Lycopersicon esculentum*) chloroplast membranes. *Plant Biology, 7(4),* 358–368. DOI 10.1055/s-2005-837696.

10. Hakmaoui, A., Ater, M., Bóka, K., Barón, M. (2007). Copper and cadmium tolerance, uptake and effect on chloroplast ultrastructure. Studies on salix purpurea and phragmites australis. *Zeitschrift für Naturforschung C, 62(5–6),* 417–426. DOI 10.1515/znc-2007-5-616.
11. Goussi, R., Manaa, A., Derbali, W., Ghnaya, T., Abdelly, C. et al. (2018). Combined effects of NaCl and Cd^{2+} stress on the photosynthetic apparatus of thellungiella salsuginea. *Biochimica et Biophysica Acta-Bioenergetics, 1859(12),* 1274–1287. DOI 10.1016/j.bbabio.2018.10.001.
12. Atal, N., Saradhi, P. P., Mohanty, P. (1991). Inhibition of the chloroplast photochemical reactions by treatment of wheat seedlings with low concentrations of cadmium: Analysis of electron transport activities and changes in fluorescence yield. *Plant and Cell Physiology, 32(7),* 943–951. DOI 10.1093/oxfordjournals.pcp.a078181.
13. Zsiros, O., Nagy, G., Patai, R., Solymosi, K., Gasser, U. et al. (2020). Similarities and differences in the effects of toxic concentrations of cadmium and chromium on the structure and functions of thylakoid membranes in chlorella variabilis. *Frontiers in Plant Science, 11,* 1006. DOI 10.3389/fpls.2020.01006.
14. Song, X., Yue, X., Chen, W., Jiang, H., Han, Y. et al. (2019). Detection of cadmium risk to the photosynthetic performance of hybrid pennisetum. *Frontiers in Plant Science, 10,* 1–14. DOI 10.3389/fpls.2019.00798.
15. Faller, P., Kienzler, K., Krieger-Liszkay, A. (2005). Mechanism of Cd^{2+} toxicity: Cd^{2+} inhibits photoactivation of photosystem II by competitive binding to the essential Ca^{2+} site. *Biochimica et Biophysica Acta-Bioenergetics, 1706(1–2),* 158–164. DOI 10.1016/j.bbabio.2004.10.005.
16. Chojnacka, K., Saeid, A., Michalak, I. (2012). The possibilities of the application of algal biomass in the agriculture. *CHEMIK–Science-Technique-Market, 66(11),* 1235–1248.
17. Suksabye, P., Pimthong, A., Dhurakit, P., Mekvichitsaeng, P., Thiravetyan, P. (2016). Effect of biochars and microorganisms on cadmium accumulation in rice grains grown in Cd-contaminated soil. *Environmental Science and Pollution Research, 23(2),* 962–973. DOI 10.1007/s11356-015-4590-8.
18. Leong, Y. K., Chang, J. S. (2020). Bioremediation of heavy metals using microalgae: Recent advances and mechanisms. *Bioresource Technology, 303,* 122886. DOI 10.1016/j.biortech.2020.122886.
19. Mohsenpour, S. F., Hennige, S., Willoughby, N., Adeloye, A., Gutierrez, T. (2021). Integrating micro-algae into wastewater treatment: A review. *Science of the Total Environment, 752,* 142168. DOI 10.1016/j.scitotenv.2020.142168.
20. Spain, O., Plöhn, M., Funk, C. (2021). The cell wall of green microalgae and its role in heavy metal removal. *Physiologia Plantarum, 173(2),* 526–535. DOI 10.1111/ppl.13405.
21. Priyadarshani, I., Sahu, D., Rath, B. (2012). Microalgal bioremediation: Current practices and perspectives. *Journal of Biochemical Technology, 3(3),* 299–304.
22. Suresh Kumar, K., Dahms, H. U., Won, E. J., Lee, J. S., Shin, H. K. (2015). Microalgae–A promising tool for heavy metal remediation. *Ecotoxicology and Environmental Safety, 113,* 329–352. DOI 10.1016/j.ecoenv.2014.12.019.
23. Ahmad, S., Pandey, A., Pathak, V. V., Tyagi, V. V., Kothari, R. (2020). Phycoremediation: Algae as eco-friendly tools for the removal of heavy metals from wastewaters. *Bioremediation of Industrial Waste for Environmental Safety,* pp. 53–76. Singapore: Springer. DOI 10.1007/978-981-13-3426-9_3.
24. Megharaj, M., Ragusa, S. R., Naidu, R. (2003). Metal-algae interactions: Implications of bioavailability. In: Bioavailability, toxicity and risk relationships in ecosystems, *4th International Symposium on the Biogeochemistry of Trace Elements,* pp. 109–144. Berkeley, California.
25. Ubando, A. T., Africa, A. D. M., Maniquiz-Redillas, M. C., Culaba, A. B., Chen, W. H. et al. (2021). Microalgal biosorption of heavy metals: A comprehensive bibliometric review. *Journal of Hazardous Materials, 402,* 123431. DOI 10.1016/j.jhazmat.2020.123431.
26. Gray, N. F. (2010). *Water technology: An introduction for environmental scientists and engineers*. Oxford: Butterworth-Heinemann.
27. Monteiro, C. M., Castro, P. M. L., Malcata, F. X. (2011). Capacity of simultaneous removal of zinc and cadmium from contaminated media, by two microalgae isolated from a polluted site. *Environmental Chemistry Letters, 9(4),* 511–517. DOI 10.1007/s10311-011-0311-9.
28. Cossich, E. S., Tavares, C. R. G., Ravagnani, T. M. K. (2002). Biosorption of chromium (III) by Sargassum sp. biomass. *Electronic Journal of Biotechnology, 5(2),* 133–140. DOI 10.2225/vol5-issue2-fulltext-4.

29. Al-qunaibit, M. H. (2004). A kinetic study of uptake of some cationic entities by the alga *Chlorella vulagaris*. *Chemindix, (12),* 1–12.
30. Perales-Vela, H. V., Peña-Castro, J. M., Cañizares-Villanueva, R. O. (2006). Heavy metal detoxification in eukaryotic microalgae. *Chemosphere, 64(1),* 1–10. DOI 10.1016/j.chemosphere.2005.11.024.
31. Cobbett, C., Goldsbrough, P. (2002). Phytochelatins and metallothioneins: Roles in heavy metal detoxification and homeostasis. *Annual Review of Plant Biology, 53(1),* 159–182. DOI 10.1146/annurev.arplant.53.100301.135154.
32. de-Bashan, L. E., Bashan, Y. (2010). Immobilized microalgae for removing pollutants: Review of practical aspects. *Bioresource Technology, 101(6),* 1611–1627. DOI 10.1016/j.biortech.2009.09.043.
33. Khan, M. I. R., Fatma, M., Per, T. S., Anjum, N. A., Khan, N. A. (2015). Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. *Frontiers in Plant Science, 6*. DOI 10.3389/fpls.2015.00462.
34. Renuka, N., Guldhe, A., Prasanna, R., Singh, P., Bux, F. (2018). Microalgae as multi-functional options in modern agriculture: Current trends, prospects and challenges. *Biotechnology Advances, 36(4),* 1255–1273. DOI 10.1016/j.biotechadv.2018.04.004.
35. Tiwari, S., Lata, C. (2018). Heavy metal stress, signaling, and tolerance due to plant-associated microbes: An overview. *Frontiers in Plant Science, 9*. DOI 10.3389/fpls.2018.00452.
36. Faheed, F. A., Abd-el Fattah, Z. (2008). Effect of *Chlorella vulgaris* as bio-fertilizer on growth parameters and metabolic aspects of lettuce plant. *Journal of Agriculture and Social Sciences, 4,* 165–169.
37. Barone, V., Puglisi, I., Fragalà, F., Lo Piero, A. R., Giuffrida, F. et al. (2019). Novel bioprocess for the cultivation of microalgae in hydroponic growing system of tomato plants. *Journal of Applied Phycology, 31(1),* 465–470. DOI 10.1007/s10811-018-1518-y.
38. Barone, V., Baglieri, A., Stevanato, P., Broccanello, C., Bertoldo, G. et al. (2018). Root morphological and molecular responses induced by microalgae extracts in sugar beet (*Beta vulgaris* L.). *Journal of Applied Phycology, 30(2),* 1061–1071. DOI 10.1007/s10811-017-1283-3.
39. Hajnal-Jafari, T., Seman, V., Stamenov, D., Uric, S. (2020). Effect of chlorella vulgaris on growth and photosynthetic pigment content in swiss chard (*Beta vulgaris* L. Subsp. Cicla). *Polish Journal of Microbiology, 69(2),* 235–238. DOI 10.33073/PJM-2020-023.
40. Chiaiese, P., Corrado, G., Colla, G., Kyriacou, M. C., Rouphael, Y. (2018). Renewable sources of plant biostimulation: Microalgae as a sustainable means to improve crop performance. *Frontiers in Plant Science, 871,* 1–6. DOI 10.3389/fpls.2018.01782.
41. Kusvuran, S. (2021). Microalgae (*Chlorella vulgaris* Beijerinck) alleviates drought stress of broccoli plants by improving nutrient uptake, secondary metabolites, and antioxidative defense system. *Horticultural Plant Journal, 7(3),* 221–231. DOI 10.1016/j.hpj.2021.03.007.
42. El-Baky, H. H. A., El-Baz, F. K., Baroty, G. S. E. (2010). Enhancing antioxidant availability in wheat grains from plants grown under seawater stress in response to microalgae extract treatments. *Journal of the Science of Food and Agriculture, 90(2),* 299–303. DOI 10.1002/jsfa.3815.
43. Dobrikova, A. G., Yotsova, E. K., Börner, A., Landjeva, S. P., Apostolova, E. L. (2017). The wheat mutant DELLA-encoding gene (Rht-B1c) affects plant photosynthetic responses to cadmium stress. *Plant Physiology and Biochemistry, 114,* 10–18. DOI 10.1016/j.plaphy.2017.02.015.
44. Rashkov, G. D., Dobrikova, A. G., Pouneva, I. D., Misra, A. N., Apostolova, E. L. (2012). Sensitivity of chlorella vulgaris to herbicides. Possibility of using it as a biological receptor in biosensors. *Sensors and Actuators B: Chemical, 161(1),* 151–155. DOI 10.1016/j.snb.2011.09.088.
45. Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology, 148,* 350–382. DOI 10.1016/0076-6879(87)48036-1.
46. Stefanov, M. A., Rashkov, G. D., Yotsova, E. K., Borisova, P. B., Dobrikova, A. G. et al. (2021). Different sensitivity levels of the photosynthetic apparatus in *Zea mays* L. and *Sorghum bicolor* L. under salt stress. *Plants, 10(7),* 1469. DOI 10.3390/plants10071469.
47. Roháček, K. (2002). Chlorophyll fluorescence parameters: The definitions, photosynthetic meaning, and mutual relationships. *Photosynthetica, 40(1),* 13–29. DOI 10.1023/A:1020125719386.

48. Stefanov, M., Yotsova, E., Markovska, Y., Apostolova, E. L. (2018). Effect of high light intensity on the photosynthetic apparatus of two hybrid lines of paulownia grown on soils with different salinity. *Photosynthetica, 56(3),* 832–840. DOI 10.1007/s11099-017-0735-y.
49. Zeinalov, Y. (2002). An equipment for investigations of photosynthetic oxygen production reactions. *Bulgarian Journal of Plant Physiology, 28,* 57–67.
50. Mesnoua, M., Mateos-Naranjo, E., Barcia-Piedras, J. M., Pérez-Romero, J. A., Lotmani, B. et al. (2016). Physiological and biochemical mechanisms preventing Cd-toxicity in the hyperaccumulator *Atriplex halimus* L. *Plant Physiology and Biochemistry, 106,* 30–38. DOI 10.1016/j.plaphy.2016.04.041.
51. Apostolova, E. L., Dobrikova, A. G., Ivanova, P. I., Petkanchin, I. B., Taneva, S. G. (2006). Relationship between the organization of the PSII supercomplex and the functions of the photosynthetic apparatus. *Journal of Photochemistry and Photobiology B: Biology, 83(2),* 114–122. DOI 10.1016/j.jphotobiol.2005.12.012.
52. Zodape, S. T. (2001). Seaweeds as a biofertilizer. *Journal of Scientific and Industrial Research, 60(5),* 378–382.
53. Tripathi, R. D., Dwivedi, S., Shukla, M. K., Mishra, S., Srivastava, S. et al. (2008). Role of blue green algae biofertilizer in ameliorating the nitrogen demand and fly-ash stress to the growth and yield of rice (*Oryza sativa* L.) plants. *Chemosphere, 70(10),* 1919–1929. DOI 10.1016/j.chemosphere.2007.07.038.
54. Kowalczy, K., Zielony, T. (2008). Effect of aminoplant and asahi on yield and quality of lettuce grown on rockwool. *Proceedings of the Conference of Biostimulators in Modern Agriculture*, pp. 7–8. Warsaw, Poland.
55. Zhang, J., Wang, X., Zhou, Q. (2017). Co-cultivation of chlorella spp and tomato in a hydroponic system. *Biomass and Bioenergy, 97,* 132–138. DOI 10.1016/j.biombioe.2016.12.024.
56. Jäger, K., Bartók, T., Ördög, V., Barnabás, B. (2010). Improvement of maize (*Zea mays* L.) anther culture responses by algae-derived natural substances. *South African Journal of Botany, 76(3),* 511–516. DOI 10.1016/j.sajb.2010.03.009.
57. Stirk, W. A., Ördög, V., Novák, O., Rolčík, J., Strnad, M. et al. (2013). Auxin and cytokinin relationships in 24 microalgal strains1. *Journal of Phycology, 49(3),* 459–467. DOI 10.1111/jpy.12061.
58. Moussa, H. R., El-Gamal, S. M. (2010). Effect of salicylic acid pretreatment on cadmium toxicity in wheat. *Biologia Plantarum, 54(2)*. DOI 10.1007/s10535-010-0054-7.
59. Maurya, R., Prasad, S. M., Gopal, R. (2008). LIF technique offers the potential for the detection of cadmium-induced alteration in photosynthetic activities of *Zea mays* L. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews, 9(1),* 29–35. DOI 10.1016/j.jphotochemrev.2008.03.001.
60. Arivazhagan, V., Sharavanan, P. S. (2015). Effect of cadmium on photosynthetic responses and biochemical contents of maize plants. *Americal Journal of Environmental Science and Engineering, 2(3),* 32–36.
61. Xue, Z. C., Gao, H. Y., Zhang, L. T. (2013). Effects of cadmium on growth, photosynthetic rate and chlorophyll content in leaves of soybean seedlings. *Biologia Plantarum, 57(3),* 587–590. DOI 10.1007/s10535-013-0318-0.
62. Stoitchkova, K., Busheva, M., Apostolova, E. L., Andreeva, A. (2006). Changes in the energy distribution in mutant thylakoid membranes of pea with modified pigment content. II. Changes due to magnesium ions concentration. *Journal of Photochemistry and Photobiology B: Biology, 83(1),* 11–20. DOI 10.1016/j.jphotobiol.2005.11.011.
63. Petrova, N., Todinova, S., Paunov, M., Kovács, L., Taneva, S. et al. (2018). Thylakoid membrane unstacking increases LHCII thermal stability and lipid phase fluidity. *Journal of Bioenergetics and Biomembranes, 50(6),* 425–435. DOI 10.1007/s10863-018-9783-7.
64. Nirmal Kumar, J. I., Oommen, C. (2012). Removal of heavy metals by biosorption using freshwater alga Spirogyra hyalina. *Journal of Environmental Biology, 33(1),* 27–31.
65. Andrade, A. D., Rollemberg, M. C. E., Nóbrega, J. A. (2005). Proton and metal binding capacity of the green freshwater alga chaetophora elegans. *Process Biochemistry, 40(5),* 1931–1936. DOI 10.1016/j.procbio.2004.07.007.
66. Mehta, S. K., Gaur, J. P. (2005). Use of algae for removing heavy metal ions from wastewater: Progress and prospects. *Critical Reviews in Biotechnology, 25(3),* 113–152. DOI 10.1080/07388550500248571.
67. Ji, L., Xie, S., Feng, J., Li, Y., Chen, L. (2012). Heavy metal uptake capacities by the common freshwater green alga cladophora fracta. *Journal of Applied Phycology, 24(4),* 979–983. DOI 10.1007/s10811-011-9721-0.

68. Guzmán-Murillo, M. A., Ascencio, F., Larrinaga-Mayoral, J. A. (2013). Germination and ROS detoxification in bell pepper (*Capsicum annuum* L.) under NaCl stress and treatment with microalgae extracts. *Protoplasma, 250(1),* 33–42. DOI 10.1007/s00709-011-0369-z.

69. Lin, X., Mou, R., Cao, Z., Xu, P., Wu, X. et al. (2016). Characterization of cadmium-resistant bacteria and their potential for reducing accumulation of cadmium in rice grains. *Science of the Total Environment, 569–570,* 97–104. DOI 10.1016/j.scitotenv.2016.06.121.

70. Jan, R., Khan, M. A., Asaf, S., Lubna, Lee, I. J. et al. (2019). Metal resistant endophytic bacteria reduces cadmium, nickel toxicity, and enhances expression of metal stress related genes with improved growth of oryza sativa, via regulating its antioxidant machinery and endogenous hormones. *Plants, 8(10)*. DOI 10.3390/plants8100363.

71. Croce, R., van Amerongen, H. (2011). Light-harvesting and structural organization of photosystem II: From individual complexes to thylakoid membrane. *Journal of Photochemistry and Photobiology B: Biology, 104(1–2),* 142–153. DOI 10.1016/j.jphotobiol.2011.02.015.

72. Chugh, L. K., Sawhney, S. K. (1999). Photosynthetic activities of pisum sativum seedlings grown in presence of cadmium. *Plant Physiology and Biochemistry, 37(4),* 297–303. DOI 10.1016/S0981-9428(99)80028-X.

73. Vassilev, A., Lidon, F., Scotti, P., Da Graca, M., Yordanov, I. (2004). Cadmium-induced changes in chloroplast lipids and photosystem activities in barley plants. *Biologia Plantarum, 48(1),* 153–156. DOI 10.1023/B:BIOP.0000024295.27419.89.

74. Sigfridsson, K. G. V., Bernát, G., Mamedov, F., Styring, S. (2004). Molecular interference of Cd^{2+} with photosystem II. *Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1659(1),* 19–31. DOI 10.1016/j.bbabio.2004.07.003.