PHOTOSYNTHETICA
International Journal for Photosynthesis Research

DOI 10.32615/ps.2025.020 PHOTOSYNTHETICA 63 (2): 182-195, 2025

The combined effect of Cd and high light stress on the photochemical processes in *Arabidopsis thaliana*

D. GIORDANO, M. BARTÁK+, and J. HÁJEK

Masaryk University, Faculty of Science, Department of Experimental Biology, Laboratory of Photosynthetic Processes, Kamenice 5, 62500 Brno, Czech Republic

Abstract

The adverse effects of cadmium on plants are accompanied by a limitation of photosynthesis, due to the production of reactive oxygen species, leading to oxidative damage to PSII and the disruption of key protein complexes involved in photosynthetic pathways. We investigated the effects of cadmium stress combined with high light in *Arabidopsis thaliana*, as dependent on the cadmium dose applied. The aim was to investigate the combined effect of the two stressors on photochemical processes with the hypothesis that Cd stress enhances the negative effect of the high light. The plants were treated with 0, 1, 10, and 50 mM Cd added as $CdCl_2$ solution to soil (potted plants), and a high light stress. The highest dose (50 mM) induced a significant oxidative stress, reduced chlorophyll fluorescence parameters related to PSII functioning and increased energy dissipation mechanisms. Elevated Cd contents impaired the electron transport and limited PSII efficiency. OJIP analysis revealed a Cd-induced K- and L-band appearance documenting LHC–PSII limitation. The combination of Cd and high light stress resulted in the photoinhibition effects in PSII, *i.e.*, a decrease in potential and effective yields of PSII.

Keywords: cadmium; chlorophyll fluorescence; heavy metal; nonphotochemical quenching; OJIP; photoinhibition; protective mechanisms.

Introduction

Plants, integral components of terrestrial ecosystems, are serving as a primary source of nutrition for both humans and animals; the well-being of plants is increasingly threatened, especially in a climate change regime, by various environmental stressors, among them, heavy metals. Cadmium (Cd) is one of the most hazardous and nonessential elements that has the potential to enter the food chain, constituting a threat to human health (Nishijo *et al.* 2017, Bharagava and Saxena 2020).

Cadmium pollution in the environment, primarily originating from anthropogenic sources, such as industrial processes, intensive agricultural practices, and the improper disposal of electronic waste (Bharagava and Saxena 2020), has led to its accumulation in soils and waters. Consequently, plants are constantly exposed to the detrimental effects of cadmium. The adverse effects of cadmium on plants are

Highlights

- High Cd (50 mM) induced a significant reduction in Chl fluorescence
- Cd exposure led to impaired electron transport rate and decreased PSII performance
- Cd- and high light stress [1,600 µmol(photon) m^{-2} s^{-1}] together increased NPQt and q_{It}

Received 7 March 2025
Accepted 18 June 2025
Published online 8 July 2025

+Corresponding author
e-mail: mbartak@sci.muni.cz

Abbreviations: ABS/RC – absorbed energy per reaction centre; DI_0/RC – dissipated energy flux per reaction centre; ET_0/RC – electron transport flux per reaction centre; ETR – electron transport rate of PSII; F_V/F_M – maximum (potential) yield of photosynthetic processes in PSII; NPQt – nonphotochemical quenching; PI_{ABS} – performance index; q_{Et} – energy-dependent quenching; q_{It} – photoinhibitory quenching; q_L – fraction of PSII centres in open states; Rfd – chlorophyll fluorescence decrease ratio; TR_0/RC – the trapped energy flux per reaction centre; Φ_{PSII} – effective quantum yield of photosynthetic processes of PSII.

Acknowledgments: The authors would like to thank the infrastructure of the VAN 2024 project (Ministry of Education, Youth and Sports of the Czech Republic) for technical and instrumental support during laboratory-based measurements. The authors are indebted to Markéta Šámalová (Masaryk University, Brno) for providing seeds of *A. thaliana* (wild type).

Conflict of interest: The authors declare that they have no conflict of interest.

wide-ranging and multifaceted, encompassing changes in growth, water and nutrient uptake, and different metabolic processes (as reviewed by El Rasafi *et al.* 2022) including photosynthesis. Cadmium uptake negatively affects root growth and leaf development; even at low concentrations, cadmium decreases dry biomass production and induces oxidative damage by causing reactive oxygen species (ROS) production (Cho and Seo 2005, Ben Ammar *et al.* 2008, Piotto *et al.* 2018).

One of the most crucial physiological processes impacted by cadmium exposure is photosynthesis, the driving force behind plant growth and biomass production (Xin *et al.* 2019, Dobrikova *et al.* 2021). The production of ROS, the oxidative damage and the disruption of pigment–protein complexes – from oxygen-evolving complex to PSII core complex to PSI – led to an overall decline in photosynthetic processes (Faller *et al.* 2005, Pagliano *et al.* 2006, Parmar *et al.* 2013, Sharma *et al.* 2020).

While the effect of cadmium on plant growth and physiology is widely studied, its combination with other stressors plays a central role nowadays. Abiotic stress, such as temperature, high light or drought, is quite common in ecosystems and agricultural systems, and the constant increase in extreme weather phenomena is leading to a growth in the intensity and severity of these stresses.

High light determines a reduction of photochemical processes (*i.e.*, photoinhibition), due to the excess of light energy that the antenna complex and pigment–protein complexes need to quench, by activating several secondary mechanisms, and an overproduction of ROS, which can induce photooxidative damage (in more severe cases of oxidative damage caused by excess of light energy, the photoinhibition becomes photodestruction) (Didaran *et al.* 2024).

Chlorophyll (Chl) fluorescence techniques have emerged as powerful tools for studying the photosynthetic efficiency of plants under various stress conditions. Our study aims to investigate the effects of cadmium stress on the model plant *Arabidopsis thaliana*, focusing on its photosynthetic response. We utilized several advanced Chl fluorescence techniques: (*1*) fast Chl fluorescence transients (OJIPs) (Strasser *et al.* 2004, for their descriptions, *see* the paragraphs below), (*2*) slow Kautsky kinetics supplemented with quenching analysis, and (*3*) induction curve of photosynthetic electron transport (ETR) in continuous constant light. The three methods were used to elucidate the mechanisms by which cadmium disrupts primary photosynthetic processes in plants and to deepen knowledge on the combined effect of short-term photoinhibition and Cd accumulation in plant tissues.

Recently, Cd effects on primary photosynthetic processes, functioning of PSII in particular, have been evaluated using a fast Chl fluorescence transient (OJIP) frequently (Faseela *et al.* 2020). The shape of the OJIP curve changes sensitively according to the strength of Cd-induced stress in PSII as well as OJIP-derived parameters. For the OJIP shape, Cd(dose)-dependent reduction of Chl fluorescence signal resulting in a flattening of the curve is reported. In OJIP-derived parameters related to PSII functioning, a decrease is reported, as shown, *e.g.*, by Zhou *et al.* (2024a). Moreover, the OJIP-derived parameter related to thermal energy dissipation (DI_0/RC) increases with increased Cd stress (Cai *et al.* 2023).

Kautsky kinetics supplemented with saturation pulses applied in light- and dark-adapted states allow the calculation of several Chl fluorescence parameters (for review *see e.g.*, Roháček 2002). In plant stress physiology studies, potential and effective quantum yields (F_V/F_M, Φ_{PSII}) are used most frequently, including evaluation of negative Cd effects on primary photosynthesis (Moustakas *et al.* 2019, Huang *et al.* 2023). Additionally, Cd-induced increase in protective mechanisms in chloroplasts has been reported, the activation of nonphotochemical quenching in particular (Küpper *et al.* 2007). Since PSII functioning decreases with the severity of Cd stress, due to inactivation of the water-splitting complex (Pagliano *et al.* 2006) and the other processes associated with photosynthetic linear electron flow (plastoquinone pool, FNR – Szopiński *et al.* 2019), activation of NPQ helps the photosynthetic apparatus cope with Cd-induced PSII overenergization and alleviation of oxidative stress caused by reactive oxygen species formation. Therefore, NPQ analysis is a crucial part of the studies focused on plant sensitivity/resistance to Cd (Han *et al.* 2020) and/or other heavy metal ions (Moustakas *et al.* 2024 for zinc).

Light-response curves of photosynthetic electron transport (ETR curves) are another Chl fluorescence technique used for assessing stress effects on photosynthetic apparatus. The essence of the method is the exposition of a leaf to a step-increased light (photosynthetic photon flux density, PPFD) with a short acclimation at each light intensity. At each PPFD level, a saturation pulse is applied to achieve peak Chl fluorescence in the light-adapted state (F_M'). The F_M' value is used for the calculation of effective quantum yield (Φ_{PSII}) and, consequently, electron transport rate (ETR). When plotted against PPFD, ETR forms light-response ETR curves that respond sensitively to environmental stressors by a flattening, *i.e.*, decreased values of ETR throughout the whole PPFD range. Such a response has been reported, *e.g.*, for salt stress (Zhao *et al.* 2025), red and blue light (Li *et al.* 2021), and photoinhibition (Barták *et al.* 2023). ETR curves have been used to evaluate heavy metals' effects (Vanhoudt *et al.* 2014 for uranium, Chen *et al.* 2016 for copper) and toxic compounds' (Tomar and Jajoo 2019) effects on plant photosynthesis. However, light-response ETR curves are not equivalent to conventional photosynthetic light-response curves (P_N *vs.* PPFD) measured by gas-exchange methods, since the ETR curves relate to PSII activity exclusively and do not evaluate the proportion of ATP and NADPH utilization in biochemical processes of photosynthesis and ATP consumption in nonphotosynthetic processes. Therefore, ETR curves typically overestimate photosynthesis when ATP is used in nonphotosynthetic processes to a substantial extent.

The findings from this study may provide valuable insights into the strategies plants employ to cope with cadmium and high light-induced stress. Ultimately,

the knowledge gained from this research is expected to contribute to a better understanding of the consequences of heavy metal pollution on plants and the development of strategies for sustainable production in contaminated areas.

Materials and methods

Plant material and growth conditions: *Arabidopsis thaliana* Col-0 seeds were kindly provided by Markéta Šámalová (Department of Experimental Biology, Masaryk University, Brno). After sterilization in 70% alcohol for 2 min, seeds were sown in a soil mixture composed of 12 parts of peat, 2 parts of sand, and 1 part of perlite, for one week. They were germinated in a greenhouse with the following parameters: temperature of 22°C with a minimum value of 17°C and maximum of 28°C; relative humidity was 60% during the day and 65% during the night; light intensity of 200 ± 10 µmol(photon) m^{-2} s^{-1} with a 14/10-h day/night photoperiod. When seedlings showed developed cotyledons (3–4 mm in diameter), they were transplanted into 80-ml plastic pots with the same soil mixture used for germination. Plants were grown in a growth chamber at a temperature of 23°C during the day and 20°C during the night, 16/8-h day/night photoperiod at a light intensity of 160 ± 10 µmol(photon) m^{-2} s^{-1}, and a relative humidity of 40 ± 10%. Water was given by subirrigation twice a week.

Cd and high-light treatments: Three weeks after transplanting, *A. thaliana* plants developed a rosette of leaves with a diameter of ~ 6 cm. At this stage, they were separated into four groups of 11 plants, and each group was treated with a different concentration of Cd, added as $CdCl_2$ solution: 0 mM (as a control, Cd0), 1 mM (Cd1), 10 mM (Cd10), and 50 mM (Cd50). The solutions were given to every pot by pipetting; a total of 15 ml of solution was given to every pot to reach the field capacity of the soil. After 24 h of Cd exposition, high light stress was induced in three plants for each group by exposing the plants to 1,600 µmol(photon) m^{-2} s^{-1} for 30 min; after 30 min of exposition to high light plants were let recover under the light intensity used for growth [160 ± 10 µmol(photon) m^{-2} s^{-1}].

Chl fluorescence measurements: We started recording chlorophyll fluorescence (ChlF) after 24 h of Cd exposure. At this time, we recorded the ETR induction curves. Kautsky kinetics was recorded together with ETR induction curves, then after high-light exposition, after 2 and 24 h of recovery from high light. Finally, OJIP curves were taken after 24 h of recovery from high light. Below is a description of the technique in detail.

Kautsky kinetics: To evaluate the effect of Cd treatments through slow Kautsky kinetics (KK) we used an *Open FluorCam* (*Photon Systems Instruments*, Drásov, Czech Republic). Parameters derived from KK are specified in the following table. Plants (eight replicates for each group of treatment) were dark-adapted for 10 min before starting the KK measurements. A single measurement started with a basal flash of weak light [0.5 µmol(photon) m^{-2} s^{-1} of photosynthetically active radiation, PAR] applied in dark-adapted leaves, this light does not activate photosynthesis and induce the basal fluorescence F_0; after 2 s a saturation pulse [3,000 µmol(photon) m^{-2} s^{-1} of PAR, 0.8 s] induced the maximum Chl fluorescence level (F_M), followed by a 27-s dark period. Then the seedlings were exposed to a 300-s-lasting actinic light (AL) period [60 µmol(photon) m^{-2} s^{-1} PAR] that activates the photosynthetic electron flow. After AL is switched on, the Kautsky effect – a rapid increase followed by a slow decline in fluorescence level – was recorded. After 300 s, a steady state of ChlF was reached and a saturation pulse was applied to induce F_M' (maximum Chl fluorescence) in the light-adapted state. After switching off the actinic light, the background ChlF (F_0') was recorded. A final saturation pulse was given then to induce F_M'' level in the dark and the KK end. Chl fluorescence parameters calculated by *FluorCam* software are listed in the text table below.

KK data were further analysed using *MS Excel 365*. The following parameters, related to nonphotochemical quenching, were calculated: NPQt, q_{It}, and q_{Et} (according to Tietz *et al.* 2017).

ETR induction curves: Electron transport rate (ETR) induction curves were measured using a *PAM 2500* fluorometer (*Heinz Walz GmbH*, Germany). To record the ETR curves, plants (three replicates for each group) were dark-adapted for 10 min, then a modified protocol exposed them to constant actinic light of 620 µmol(photon) m^{-2} s^{-1} for 10 min with repetitive saturating pulses (each 60 s) to measure F_M' and evaluate Φ_{PSII}. During this period,

List of parameters derived from the slow Kautsky kinetic supplemented with saturation pulses. Formulae to calculate the parameters are presented in Supplementary materials.

Parameter	Meaning	Reference
F_V/F_M	Maximum (potential) yield of photosynthetic processes in PSII	Genty *et al.* (1989)
Φ_{PSII}	Effective quantum yield of photosynthetic processes of PSII	Genty *et al.* (1989)
Rfd	Chlorophyll fluorescence decrease ratio	Lichtenthaler *et al.* (2005)
q_L	Fraction of PSII centres in open states	Kramer *et al.* (2004)
NPQt	Nonphotochemical quenching	Tietz *et al.* (2017)
q_{It}	Photoinhibitory quenching	Tietz *et al.* (2017)
q_{Et}	Energy-dependent quenching	Tietz *et al.* (2017)

repetitive saturating pulses were applied and ETR levels were calculated from Φ_{PSII} values using the following equation:

$$ETR = \Phi_{PSII} \times PFD \times 0.84/2$$

where Φ_{PSII} is the effective quantum yield of PSII, PFD is the photon flux density, 0.84 reflects the fraction of incident photons absorbed by Chl *a* molecules in PSII and the division by 2 is due to the equal fraction of photons absorbed by PSII and PSI (Krall and Edwards 1992).

Finally, the maximum values along the ETR curves were taken for each treatment to display the ETR_{max}.

OJIP curves: To evaluate responses of PSII to a short-term Cd treatment combined with photoinhibition, fast kinetics OJIP were recorded using a *PAR-FluorPen* portable fluorimeter (*Photon Systems Instruments*, Drásov, Czech Republic) equipped with detachable leaf clips. A total of eight *A. thaliana* leaves for each group of treatment were dark-adapted for 10 min using the fluorimeter leaf clips, then ChlF was induced by the fluorimeter, producing a saturating light pulse of 3,000 μmol(photon) m^{-2} s^{-1}, and the fast rise of Chl fluorescence was recorded for 2,000 ms using the fluorimeter's OJIP protocol, as described by Strasser *et al.* (2004). Data were processed using *FluorPen* software and then further analysed using *MS Excel 365*.

Statistical analysis: Statistically significant differences in the Chl fluorescence parameters were evaluated by one-way analysis of variance (*ANOVA*) and *Tukey*'s test; the *P*-value ≤0.05 was considered statistically significant. Data elaboration was performed using *MS Excel* (*MS Office 365*), while statistical analysis and data visualization were conducted using scripts written in the *Python* programming language.

Results

Photosynthetic electron transport rate induction curves: The electron transport rate (ETR) indicates the ratio of electron transport through the electron chain at a given light intensity (White and Critchley 1999). Cd exposition led to a decrease in the ETR values, as shown in Fig. 1, which displays the maximum values of $ETR:ETR_{max}$. The heavy metal-induced decline of the ETR is pronounced with increased Cd concentration, but was not visible with a solution concentration of 1 mM (in our data, rather an increase than a decrease was apparent).

Kautsky kinetics: This experimental setup enabled us to detect and evaluate the effect of Cd stress, its combination with high light stress, and its related fluctuations in ChlF parameters. Cd effect on ChlF was apparent after 24 h of exposure to the heavy metal. Fig. 2*A* shows the KK related to the treatments after 24 h of Cd exposition (but before photoinhibition); these curves show the whole Kautsky effect and the ChlF levels at each moment of the kinetics. An indicator for several types of stress on primary photosynthesis was the flattening of this curve; this was evident as regards the Cd50 group.

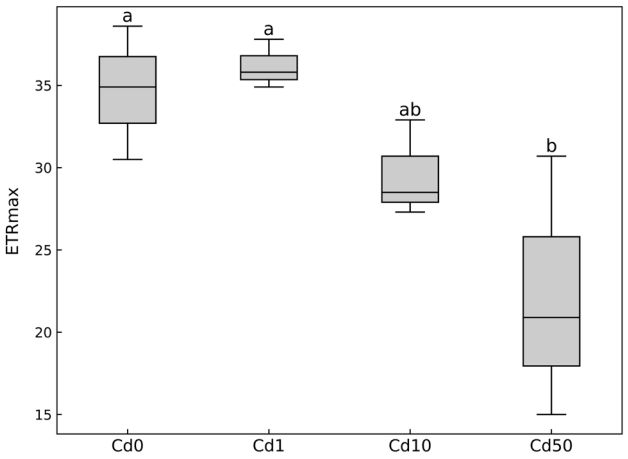

Fig. 1. Maximum electron transport rate values of *Arabidopsis thaliana* leaves under Cd stress. A total of three replicates were used for this analysis; *the different letters* above the boxes represent the significant differences between the treatments (by one-way *ANOVA* and *Tukey*'s post hoc test). Cd0 are plants treated with 0 mM $CdCl_2$ (control), Cd1, Cd10, and Cd50 are plants treated with 1, 10, and 50 mM, respectively, of $CdCl_2$.

F_P is the maximum ChlF level reached when the actinic light is switched on; after this point, there is a decline in ChlF level due to the quenching mechanisms that are activated – the Kautsky effect. Fig. 2*B* represents the curves normalized in F_P – each point was divided by the F_P value. By normalizing, in F_P is possible to better evaluate the differences in quenching mechanism after the actinic light is switched on. We expected a flattened KK curve for stressed plants; moreover, in FP-normalized curves, we expected a higher level of ChlF after the F_P point, which is an indication of a negative effect in the redox state of the plastoquinone pool. This difference was seen in the area indicated by the arrow in Fig. 2*B*: the area between the control curve and the Cd-treated ones showed the difference in how fast the quenching of the ChlF emission was; according to the graph, the area increased with the increasing Cd concentration.

From the KK it is possible to derive several parameters that illustrate various aspects of the primary photosynthetic processes. Fig. 3 shows parameters derived from the KK: F_V/F_M is the maximum quantum yield and indicates the potential photosynthetic efficiency; Φ_{PSII} represent the effective quantum yield; these parameters decrease due to the Cd stress in the first 24 h of exposure, as seen in Fig. 3 (before PI, *i.e.*, before photoinhibition). Fig. 4 displays the parameter q_L, which level indicates the redox state of the plastoquinone (PQ) pool, which is also the fraction of PSII centres in open state; and Rfd, which indicates the ChlF decrease ratio and is correlated with the net photosynthesis. These parameters followed a similar pattern: the highest dose of Cd (50 mM) caused a more rapid decline in photosynthetic performance, but even if the differences were not significant for lower doses, a progressive decrease was apparent with increasing Cd concentration.

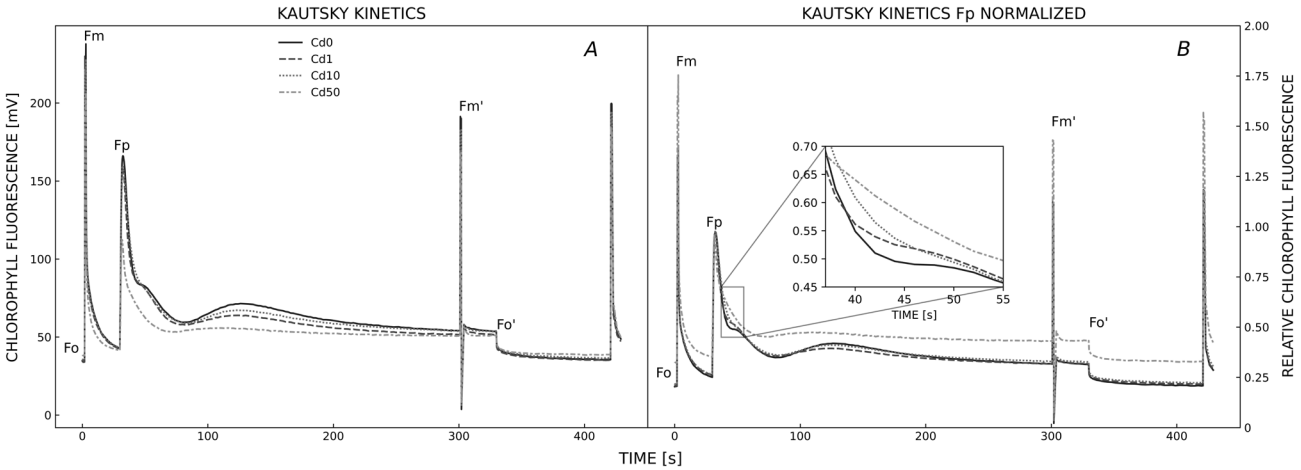

Fig. 2. Slow Kautsky kinetics of chlorophyll fluorescence (means of eight replicates) recorded on *Arabidopsis thaliana* under Cd exposition. (*A*) Kinetics without normalization; (*B*) kinetics normalized in F_P with one specific region of the curve zoomed in to better show the differences. Cd0 are plants treated with 0 mM $CdCl_2$ (control), Cd1, Cd10, and Cd50 are plants treated with 1, 10, and 50 mM, respectively, of $CdCl_2$.

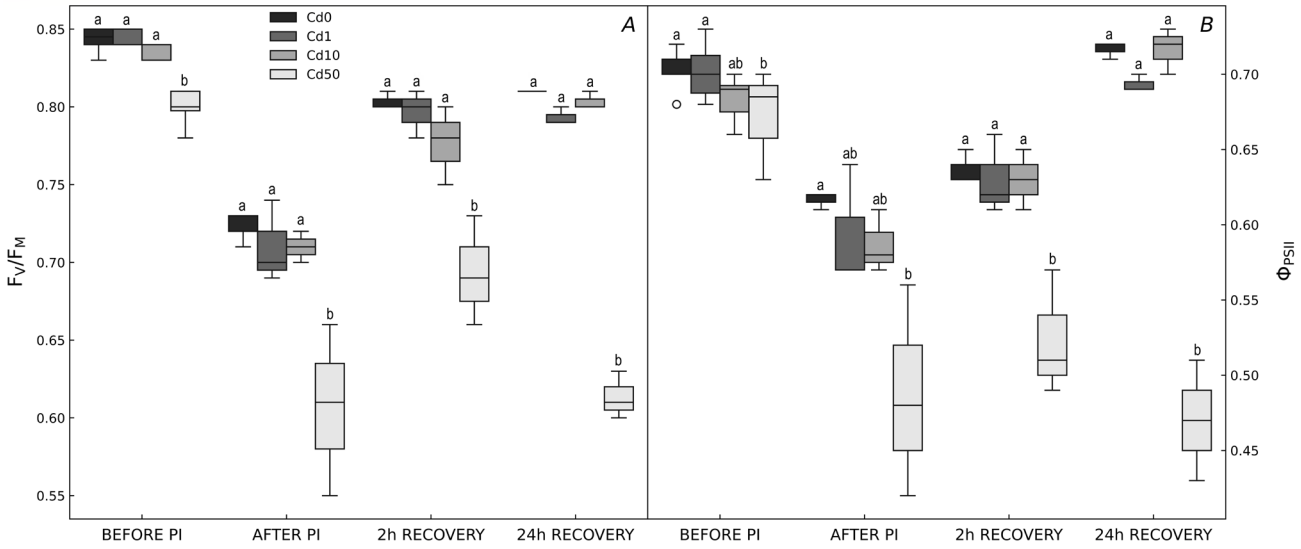

Fig. 3. Time courses of potential yield of photosynthetic processes in PSII (F_V/F_M), and effective quantum yield of photosynthetic processes in PSII (Φ_{PSII}) recorded in *Arabidopsis thaliana* affected by Cd stress before (before PI), immediately after the high light (photoinhibitory) treatment (after PI), and after 2 and 24 h of recovery. A total of eight replicates were used for this analysis; *the different letters* above the boxes represent the significant differences between the Cd treatments (by one-way *ANOVA* and *Tukey*'s post hoc test). Cd0 are plants treated with 0 mM $CdCl_2$ (control), Cd1, Cd10, and Cd50 are plants treated with 1, 10, and 50 mM, respectively, of $CdCl_2$.

From ChlF data recorded for the experimental plants, it is possible to determine the parameters related to particular pathways contributing to overall nonphotochemical quenching using the equation by Tietz *et al.* (2017). In this concept, nonphotochemical quenching (NPQt) is formed by q_{It} and q_{Et} (Fig. 5). NPQt was activated by the Cd-induced stress, as we can see in the rise of NPQt levels in Cd-treated plants (before PI). The q_{It} component – photoinhibitory quenching – plays a dominant role in this case, as it composes the major part of the NPQt, against the q_{Et} – energy-dependent quenching – that remains constant in all the treatments and follows the same pattern of the NPQt.

High light exposition effect on the photosynthesis of plants was successfully sensed with the KK analysis: parameters such as F_V/F_M and Φ_{PSII} showed a decline after 30 min of exposition to light (Fig. 3). This is because the photoinhibition induction affects the functioning of the PSII and reduces CO_2 fixation rate as well. After the exposition to high light, the two parameters recovered within a 24-h time range. The recovery pattern, however, differed between F_V/F_M and Φ_{PSII}. F_v/F_M reached a plateau after 2 h of recovery, and this was not seen in Φ_{PSII}. F_V/F_M reached the optimum values in a shorter time compared to Φ_{PSII}.

Concerning differences between Cd treatments, Cd50 plants showed significantly lower values for F_V/F_M and Φ_{PSII}; after 2 h of recovery, the values started declining again, probably because the Cd was continuing to affect the overall plant homeostasis. Plants

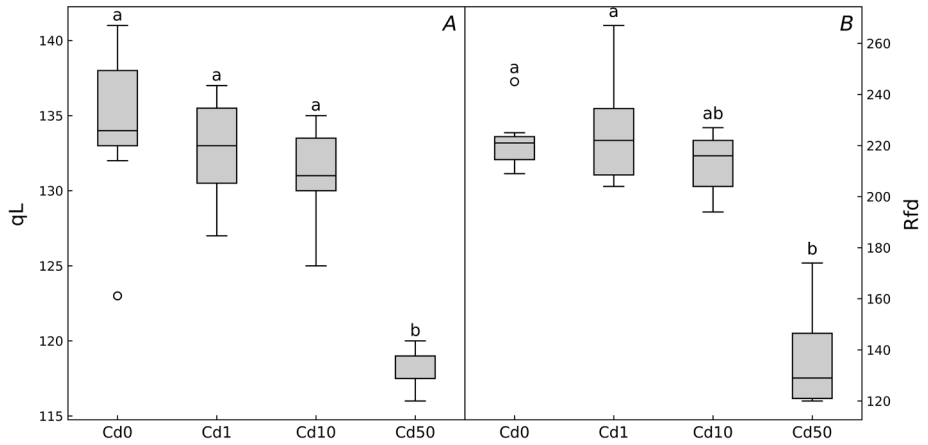

Fig. 4. Levels of ChlF parameters of *Arabidopsis thaliana* under Cd exposition. (*A*) The fraction of PSII centres in open states, q_L; (*B*) the chlorophyll fluorescence decrease ratio, Rfd. A total of eight replicates were used for this analysis; *the different letters* above the boxes represent the significant differences between the Cd treatments (by one-way *ANOVA* and *Tukey*'s post hoc test). Cd0 are plants treated with 0 mM $CdCl_2$ (control), Cd1, Cd10, and Cd50 are plants treated with 1, 10, and 50 mM, respectively, of $CdCl_2$.

treated with lower doses, Cd10 and Cd1, did not show significantly different values compared to the control. In contrast, they still appeared slightly lower than the control; this can probably mean that these doses of Cd could affect physiology, but this is not visible in the first 48 h of its exposition.

Nonphotochemical quenching also displayed a typical behaviour (*see* Fig. 5); NPQt increased when plants were affected by photoinhibition and was reduced after recovery (Fig. 5*A*). A similar pattern was observed in q_{It} (Fig. 5*B*). Also, in this case, as shown in quantum yield parameters (F_V/F_M and Φ_{PSII}), in Cd50 plants, NPQt and q_{It} were significantly higher and started to increase again during recovery. q_{Et} showed an increase during photoinhibition and then a decline during recovery (Fig. 5*C*). In Cd50 plants, it reached a rather small decrease, most probably because overall nonphotochemical quenching was increasing, keeping this parameter quite stable; overall, there were no differences in q_{Et} among groups of treatment. In general, the control group still showed a lower increase rate in NPQt and q_{It}, and a faster recovery rate, as shown in Fig. 5*B* (q_{It}), even if it was not statistically significant. Treatments with Cd appear to show a higher photoinhibitory effect compared to the control.

OJIP curves: Fig. 6*A* displays the polyphasic rise of ChlF transients from plants after 24 h of recovery from high-light exposition (48 h of Cd stress). The curves show a slight difference between groups in the O and J phase (photochemical phase): control plants show the lowest values in this phase, while they rise in plants treated with Cd. Some differences also appear in the I phase (thermic phase). The P phase shows lower values in Cd-treated plants compared to the control; this is far more accentuated in the Cd50 plants, which display a flattened curve typical for stressed plants (Kalaji *et al.* 2014). Fig. 6*B* shows the curves normalized in the O and P points; this normalization allows the enhancement of the difference in the middle phase of the curve. Cd-treated plants showed higher values compared to the control, which means that the redox state of the PQ pool was affected.

To further elucidate the differences in the ChlF transient, we double normalized the curves between 0 and 300 μs and between 0 and 2 ms, and we obtained, respectively, the K-band, between O and J steps, and the L-band, between O and K steps (Fig. 7). The K-band indicates the inactivation of the oxygen-evolving complex (OEC); the L-band can be interpreted as an indicator of connectivity loss of the three-part complex: antenna–LHC–reaction centre. Cd50 plants showed a positive band, both for K and L. In contrast, other treatments did not show an observed difference in both K and L bands compared to the control.

Fig. 1S (*supplement*) represents a radar graph that shows the parameters calculated within the transient OJIP. Before plotting, they were normalized according to the control values; Cd50 was significantly different in all the parameters. Although the differences between Cd1, Cd10, and control were not significant, these groups followed a gradual pattern from control to Cd10. Cd treatments seemed to lower the values of these parameters and increase the values of the ones connected to energy dissipation (Φ_{D0}). The performance index (PI_{ABS}) represents the performance of electron flow through the PSII expressed on an absorption basis; this parameter is quite sensitive in detecting changes in PSII functionality. Cd treatments progressively lowered the PI_{ABS}; Cd1 and Cd10 were significantly different from both control and Cd50, and the latter was the lowest and significantly different from control.

The parameters of energy flux per reaction centre – ET_0/RC, TR_0/RC, ABS/RC, DI_0/RC (Fig. 8) – delineate the distribution of absorbed light energy into various flux components responsible for driving photochemical reactions. There was no statistical difference between groups apart from the Cd50 plants; this group showed an increased value of absorption (ABS/RC), trapping (TR_0/RC), and energy dissipation (DI_0/RC) while electron transport above the plastoquinone pool (ET_0/RC) was reduced.

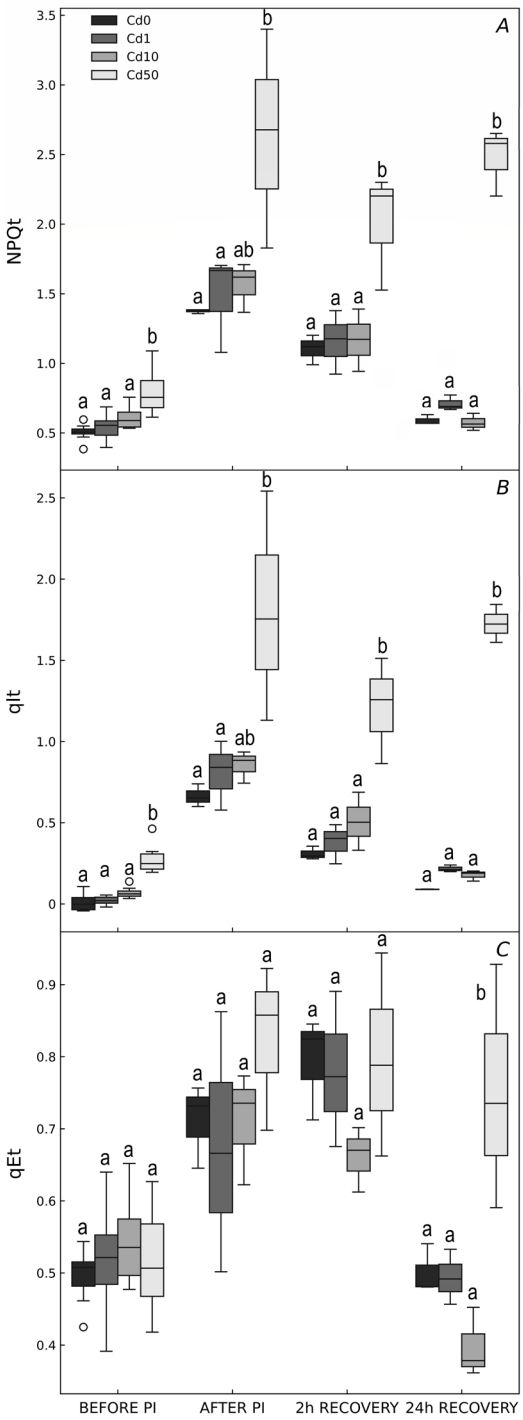

Fig. 5. Time courses of nonphotochemical quenching, NPQt (*A*), photoinhitory quenching, q_{It} (*B*), and energy-dependent quenching, q_{Et} (*C*) of chlorophyll fluorescence showing involvement of photoprotective mechanisms under Cd stress and high light stress. The NPQt, q_{It}, and q_{Et} values were evaluated before (before PI), after 30 min of high light (photoinhibitory) treatment (after PI), and after 2 and 24 h of recovery. A total of eight replicates were used for this analysis; *the different letters* above the boxes represent the significant differences between the Cd treatments (by one-way *ANOVA* and *Tukey*'s post hoc test). All parameters were calculated according to the formula by Tietz *et al.* (2017).

Discussion

Cd accumulation in soil and root uptake is complex and regulated by many factors (Alloway 2013). The most relevant are the chemical mechanisms regulating its availability in soil, such as ion chelation (Song *et al.* 2017, Liu *et al.* 2018). In this study, plants showed a dose-dependent response to Cd stress. Exposure to 50 mM of Cd caused the most evident effects on photosynthesis and plant growth, while exposure to 1 or 10 mM showed mild/hidden effects, observable after 48 h through changes in PI_{ABS} levels (Fig. 8). Cd induces diverse effects on plant physiological processes on a molecular and metabolic level (DalCorso *et al.* 2010, Lin and Aarts 2012). Among them, phytochelatin production is the main protagonist (Ben Ammar *et al.* 2008), serving for heavy-metal ion-binding by chelatinization and allocation in the vacuole (Peng and Gong 2014). Cd accumulation in plant tissue is seen to determine lipid peroxidation and enhanced H_2O_2 production, consequently, oxidative damage and a reduced electron transport chain (Cho and Seo 2005, Ben Ammar *et al.* 2008). Some of these effects could be detectable by Chl fluorescence. In this study, it proved to be a functional tool for understanding the effects of the combination of Cd stress and photoinhibition from high light exposure.

Basic chlorophyll fluorescence parameters: As seen in the reduction of F_V/F_M, Φ_{PSII}, and Rfd, Cd stress affected the primary photosynthetic processes. In Cd50 plants, a significant decrease in F_V/F_M values indicates inhibition of several processes in PSII induced by a high Cd dose. Such a response is reported for a wide variety of photosynthetic organisms, ranging from cyanobacteria (Zhou *et al.* 2006), algae (Hernandez 2016), lichens (Maslać *et al.* 2016), mosses (Bellini *et al.* 2020), ferns (Deng *et al.* 2014), to vascular plants (Pagliano *et al.* 2006). Underlying mechanisms causing a decrease in F_V/F_M are negative effects on the oxygen-evolving complex (OEC) also known as the water-splitting complex (WSC) (Linger *et al.* 2005), and oxidative destruction of PSII core proteins, D1 in particular (Zsiros *et al.* 2020). Our data support the Cd-induced negative effect of Cd on OEC, since the parameter F_V/F_0 (the efficiency of the OEC on the donor side of PSII) declined in Cd-treated plants (Fig. 1S), similarly to the data reported by Dobrikova *et al.* (2021) for Cd-treated *Salvia sclarea*.

The Cd-induced decrease in F_V/F_M is typically accompanied by an increase in protective mechanisms, nonphotochemical quenching in particular (Waheed *et al.* 2025), which was found in our study as well. The F_V/F_M decrease might be attributed to F_0 increase, F_M decrease or both. In our study, the decline in F_V/F_M could be due to an F_0 increase and might be attributed to the physical interaction of LHCII with the PSII antenna complex (Singh *et al.* 2023). These data suggest that the main affected parts of the photosynthetic apparatus are the water-splitting complex, the PSII components (LHC and PSII proteins), and the connectivity between PSII and the PQ pool. The latter explanation might be supported by the study of Todorenko *et al.* (2021), who reported

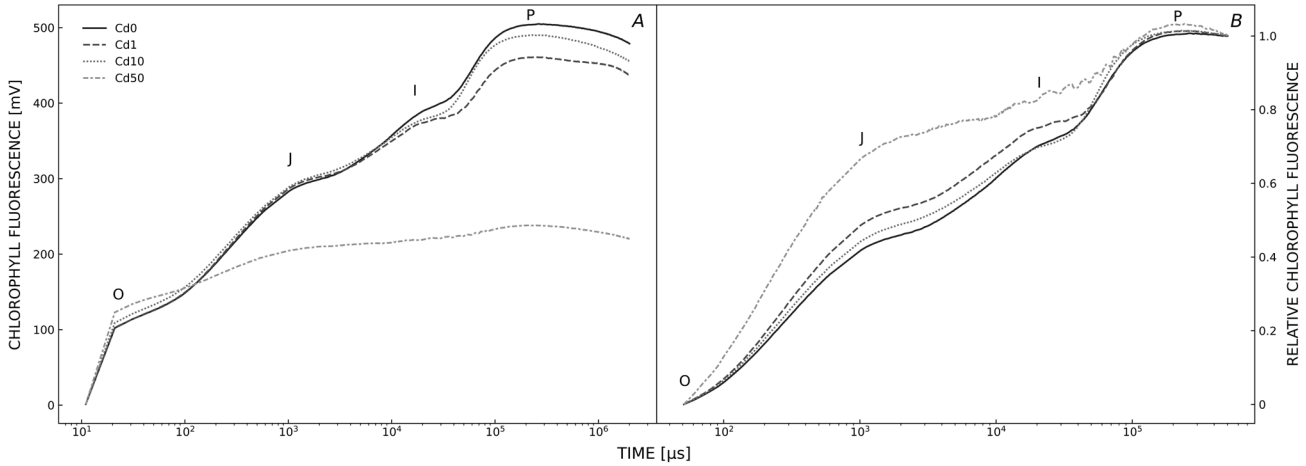

Fig. 6. Transient OJIP taken after 24 h of high-light exposition. (*A*) The standard OJIP curves; (*B*) the curves normalized in O and P points. The *x*-axis is represented as a logaritmic scale. Data points represent the means of eight replicates. Cd0 are plants treated with 0 mM $CdCl_2$ (control), Cd1, Cd10, and Cd50 are plants treated with 1, 10, and 50 mM, respectively, of $CdCl_2$.

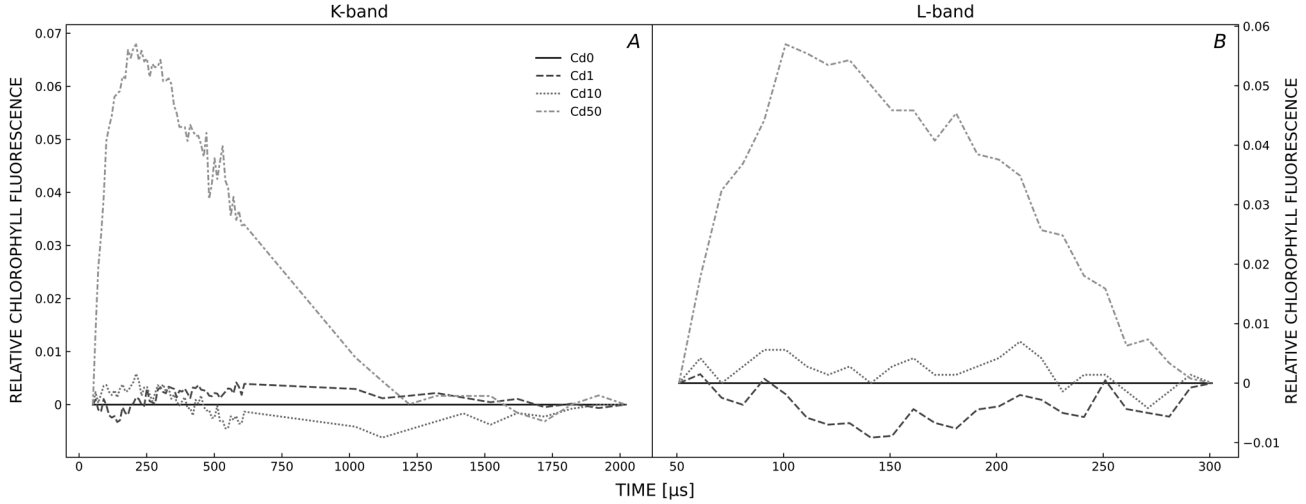

Fig. 7. Double normalized curves of K-band (*A*) that display the changes in O–J phase relative variable fluorescence intensity of Cd-treated plants in comparison with the control (Cd0); L-band (*B*) that shows the changes in O–K phase relative variable fluorescence intensity of Cd-treated plants compared with the control (Cd0). Cd0 are plants treated with 0 mM $CdCl_2$ (control), Cd1, Cd10, and Cd50 are plants treated with 1, 10, and 50 mM, respectively, of $CdCl_2$.

Cd-induced inhibition of electron transport from PSII to the PQ pool and between PQs and PSI. However, Cd-induced limitation of PSII functioning is more serious than PSI, since activation of cyclic electron transport protecting PSII has been reported (Wang *et al.* 2022a). These Cd-induced negative changes in the transfer of energy from the PSII reaction centre to electron acceptor molecules decrease PSII functioning, leading to the degradation of the PSII reaction centre if the Cd dose is too high. For *A. thaliana*, such response of F_V/F_M has been reported for the Cd concentration ranging between 50–100 µmol (Maksymiec *et al.* 2007, Martínez-Peñalver *et al.* 2012). Cd stress decreases the electron transport rate above the Q_A, generating an excess of energy at the antenna level. This generates more energy dissipation and an increase in NPQ and q_I, as it has a photoinhibitory effect. As reported by Wodala *et al.* (2012), Cd-induced stress is shown to decrease the ETR values, our results are in accordance with this as seen in Fig. 1. In general, Cd stress significantly affected PSII structural and functional activity in *A. thaliana*, which agrees with earlier research showing that higher accumulation of Cd reduces the PSII structural ability and functional activity. The Cd effects are interpreted as stress damages in the PSII antenna and PSII core, resulting in a decrease in Φ_{PSII} and impaired ETR, as has been observed by Manzoor *et al.* (2022) and our study as well.

OJIPs and OJIP-related parameters: Flattening of OJIP in terms of heavy metal-induced decrease in Chl fluorescence values forming the OJIPs is described as a consequence of heavy metal negative effects on PSII and reported for Cu (Singh *et al.* 2022), and is well documented in our data. A decline in PSII functioning (*see* an increase/

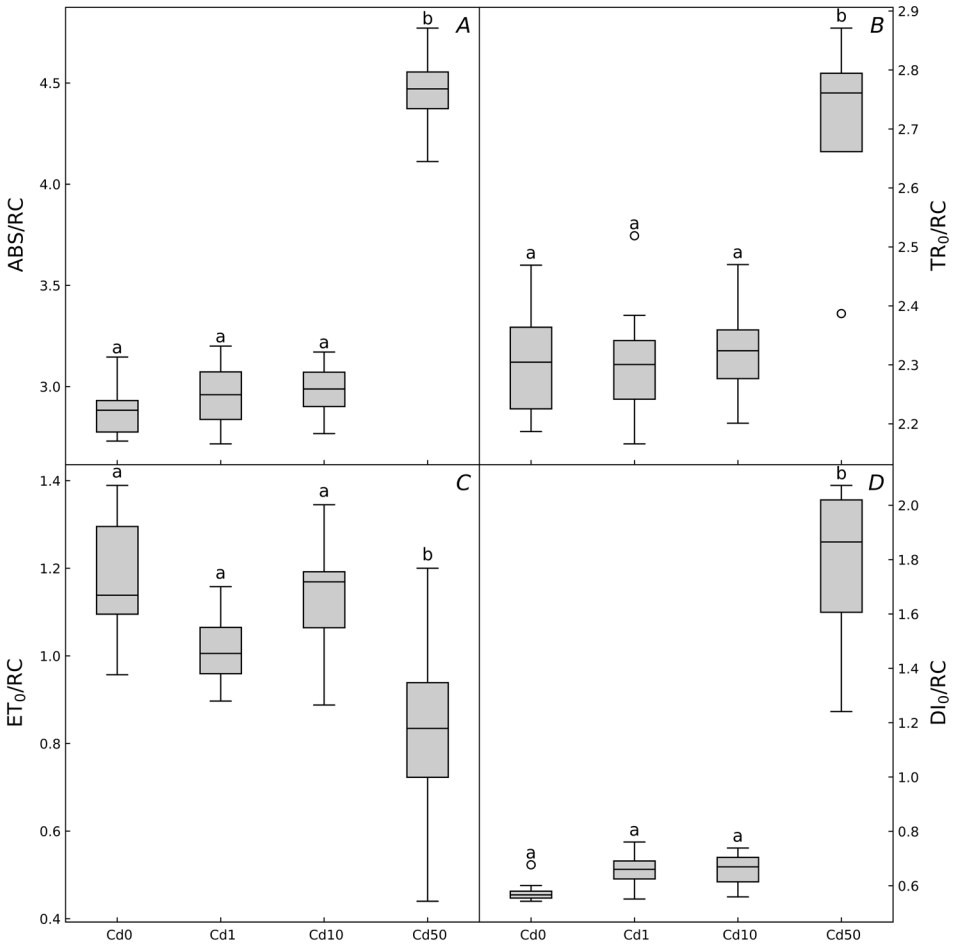

Fig. 8. Energy cascade parameters calculated within the JIP-test. ET_0/RC (*A*) represents the electron transport above Q_A^-, TR_0/RC (*B*) is the trapped reduction energy flux per reaction centre, ABS/RC (*C*) is the adsorbed energy per reaction centre, DI_0/RC (*D*) represents the total dissipated energy per reaction centre (Bussotti *et al.* 2012). *The different letters* above the boxes represent the significant differences between the treatments (by one-way *ANOVA* and *Tukey*'s post hoc test). Cd0 are plants treated with 0 mM $CdCl_2$ (control), Cd1, Cd10, and Cd50 are plants treated with 1, 10, and 50 mM, respectively, of $CdCl_2$.

decrease of OJIP-derived parameters, *see* Fig. 1S, Fig. 8) caused by Cd ions found in our study is well comparable to the evidence reported for Cd-treated plants (Faseela *et al.* 2020). The negative changes to PSII are reflected in the decrease in PI_{ABS}, an increase in DI_0/RC and consequent increase in Φ_{D0} (Fig. 8). These effects are in accordance with a recent study of Cd-treated tobacco (Cai *et al.* 2023), tomato (Chtouki *et al.* 2021), and lettuce (Chen *et al.* 2022), which report Cd concentration-dependent decrease in PI_{ABS}. This is associated with a Cd-induced decrease in PSII functioning, especially the effectiveness of energy flow through reaction centres of PSII, as well as decreased capacity of electron transmission between photosystems (Wen *et al.* 2005). Such changes are accompanied by an increase in Cd-induced thermal dissipation (DI_0/RC), as shown by *e.g.*, Kalaji and Loboda (2007) for barley, as well as the effectiveness of this process (Φ_{D0}), as a consequence of Cd-induced overenergization of PSII due to reduced photosynthetic electron transport and reported in other Cd-treated plants (*e.g.*, Cai *et al.* 2023). Cd stress caused a reduction of the electron transport from Q_A to Q_B, which is indicated by a larger trapping but a lower electron transport over the Q_A (TR_0/RC, ET_0/RC). This finding is in accordance with previous studies (Geiken *et al.* 1998).

An increase in DI_0/RC is considered a protective mechanism activated in the early stage of plant response to Cd treatment. Increased DI_0/RC helps alleviate the formation of reactive oxygen species. However, if the Cd stress lasts too long (in terms of days) or if Cd doses are too high, formation of reactive oxygen species (ROS) becomes accelerated and may lead to severe Cd-induced oxidative stress in PSII and pigment–protein complexes in the thylakoid membrane (Bi *et al.* 2009). For *A. thaliana*, however, some Cd-tolerant mutants have been created that exhibit a high synthesis of antioxidative enzymes. Such mutants show an increased (compared to wild type) tolerance to Cd-induced oxidative stress in the photosynthetic apparatus (Radeva *et al.* 2010).

Functioning of LHC–PSII complex components in Cd-treated plants is well reflected by the presence of L-band and K-band in Cd50 treatment (Fig. 7). The approach of L- and K-band is widely applied in

the research focused on primary photosynthesis in plants exposed to a wide variety of stressors, including Cd treatment (*see e.g.*, Zhou *et al.* 2024a,b). In the study, the presence of L- and K-band was reported even after 3-d exposure to 100 μmol Cd. Positive values of K- and L-bands found for Cd-treated plants (Cd50) might be attributed to excessive Cd-induced damages to the donor side of PSII and connectivity between LHCs and PSII. In majority of studies exploiting K- and L-band occurrence in response a Cd stress (*e.g.*, Wang *et al.* 2022b, Cai *et al.* 2023), concentrations of about 100 μM L^{-1} are reported to induce an increase in K-band and interpreted as a disruption of the functional link between OEC and PSII (Jiang *et al.* 2006). Similarly to these and our results, several studies (*e.g.*, Gururani *et al.* 2013, Cai *et al.* 2023, Zhou *et al.* 2024b) report that the appearance of K- and L-bands is a consequence of PSII limitation on the donor side of PSII in heavy metal-treated plants. Our data suggest that negative changes to connectivity between PSII components (L-band presence), *i.e.*, grouping the PSII units or energetic connectivity between antenna and PSII RCs (Guo *et al.* 2020), are apparent only in 50 mM Cd treatment but not in lower Cd concentrations. The same is evident for the limitation of the oxygen-evolving complex functioning (K-band presence), found exclusively in the Cd50 treatment.

An increase in ABS/RC and TR_0/RC in the Cd50 plants (Fig. 8) might be attributed to PSII behaviour under strong stress, since such a response has been documented for a wide variety of stressors, such as *e.g.*, low (chilling) temperature (Krüger *et al.* 2014), desiccation (Bednaříková *et al.* 2020), and elevated temperature combined with high light (Wang *et al.* 2022c). Specifically for heavy metals, Cu- and Hg-induced increase in ABS/RC and TR_0/RC is reported (Singh *et al.* 2023).

In the majority of plants, Cd stress leads to inhibition of photosynthetic linear electron transport chain (ET_0/RC in OJIP analysis, *see* decrease at Cd50, Fig. 8). This is well documented for a great variety of plants and cyanobacteria (Verma and Prasad 2021, Singh *et al.* 2022); however, Cd-induced decrease in ET_0/RC is associated with the reoxidation of reduced Q_A through electron transport. However, the parameter (ET_0/RC) reflects only active reaction centres. Such limitation of PSII-related photosynthetic processes is associated with an increase in thermal dissipation DI_0/RC (Fig. 8, Cd10), which is considered a protective mechanism activated in stressed and less efficiently working PSIIs. Such Cd-induced response is well described and associated with an extra load of still active RCs (Gonzalez-Mendoza *et al.* 2007).

Combination with high light stress: Excess light energy led to a bigger production of ROS and consequently oxidative damage. Several tolerance mechanisms are activated in plants in response to high light stress, such as the xanthophyll cycle and other cycles that lead to an enhanced level of nonphotochemical quenching (Sharma *et al.* 2023).

The effect of the combination of Cd and high light stress was Cd dose-dependent. Cd stress increased photoinhibitory effects caused by high light only in Cd50 treatment. In contrast, the lower Cd concentrations did not affect PSII functioning in comparison with untreated control during photoinhibition and consequent 48-h recovery (*see* F_V/F_M and Φ_{PSII} in Fig. 3). This suggests that effective Cd concentration causing inhibition of PSII lies between 10–50 mM Cd which is comparable to data reported by Gharbi *et al.* (2018) for *Solanum lycopersicum* due to high Cd content in leaves; the effective concentration might be, however, one or two order of magnitude lower in microalgae (Faller *et al.* 2005).

In our study, NPQt, its q_{It} component in particular, increased in the Cd50 plants after the short-term photoinhibitory treatment (Fig. 5), indicating the co-action of high light and heavy metal stress in chloroplasts and activation of photoprotective mechanisms. In vast majority of plants, nonphotochemical quenching increases in response to a short-term photoinhibition as documented for algae (Wang *et al.* 2022d), lichens (Haq *et al.* 2024), mosses (Orekhova *et al.* 2021), and vascular plants having C_3 (Hikosaka 2021), C_4 (Shay and Kubien 2013), alternative C_3/CAM (Matsuoka *et al.* 2018), and CAM (Wang *et al.* 2022e) carbon-fixing mechanism. The photoinhibition-induced increase in NPQ has been reported for *A. thaliana* as well and, similarly to our study (*see* Fig. 5, q_{It}), attributed to an increase in photoinhibitory quenching (Jin *et al.* 2014). The q_I increase is caused by high light-induced structural and functional changes in LHCII–PSII supercomplex (Malnoë 2018). The q_I component of nonphotochemical quenching is mainly related to inactivation or even damage of the reaction centres of PSII, recognized as the slowest relaxing component requiring hours to recover (Lichtenthaler 2005), *i.e.*, to achieve pre-photoinhibition level. Together with fast-activated and fast-relaxed as well, energy quenching (q_E, seconds to minutes), q_I represents an effective photoprotective mechanism. In short-term photoinhibition, fast-activated photoprotective mechanisms play a significant role in the initial response of the chloroplast apparatus to high light. Among them, q_E, which is associated with high light-induced ΔpH-dependent conversion of violaxanthin to zeaxanthin (for *A. thaliana see e.g.*, Wei *et al.* 2024), plays an important role. Apart from zeaxanthin formation, PsbS (Li *et al.* 2000) and lutein (Pogson *et al.* 1998) are associated with q_E. However, some studies suggest that q_E-type quenching can be induced only by artificially lowered lumenal pH and resulting acceleration in cyclic electron flow (Johnson and Ruban 2011, Johnson *et al.* 2012).

The reduction of electron transport and the increase in thermal dissipation are the two key mechanisms behind Cd stress response (Cai *et al.* 2023); what is seen after the induction of high light stress could suggest that mechanisms other than the ones activated in response to Cd are activated in response to high light stress. The more rapid increase in q_{It} and decline in Φ_{PSII} found immediately after the photoinhibitory treatment indicate that the primary photosynthetic metabolism is more susceptible to a surplus of incident light when already exposed to a stress

such as Cd, which slows down the electron flow at the Q_A level and oxygen-evolving complex.

The negative effect of Cd on photosynthetic parameters as reported in our study is typical for high Cd content in leaves, which is species-specific, however, ranging from 0.6 to 9.1 mg kg^{-1} (Baldantoni *et al.* 2016). Cd uptake and transport from roots to shoots is nonspecific, because of the great number of metal transporters and nonselective ion channels which are involved. Multiple factors, such as *e.g.*, root uptake, sequestration in root vacuoles, translocation through xylem and phloem, and dilution during plant growth, may influence the accumulation of Cd in plant shoots. Moreover, even Cl ions released from $CdCl_2$ used in our study may interact with the negative effect of Cd on PSII-related chlorophyll fluorescence parameters; however, such interaction might be neglected, similarly to other studies exploiting the addition of $CdCl_2$ to cultivation medium/substrate (Naciri *et al.* 2024). Phytochelatins, which are synthesized both in shoots and roots in dependence on the severity of Cd stress (Mou *et al.* 2016), may influence Cd allocation in particular plant tissues and photosynthesis. They are biomolecules acting against the toxic effects of Cd and other heavy metal ions in plants (for review *see* Merlos Rodrigo *et al.* 2016 and Faizan *et al.* 2024). They affect the extent of Cd complexation in different plant parts by forming Cd–phytochelatin complexes that sequester the Cd and therefore lessen the toxic effects. Thus, allocation of Cd rather in shoots and/or roots is affected by phytochelatin synthesis and allocation. Therefore, the differences in Cd allocation represent an important factor since they affect the amount of Cd which is found in leaves and the chloroplasts. Moreover, subcellular Cd localization in shoot and root cells (Van Belleghem *et al.* 2007) plays an important role since it may affect the effective amount of Cd in the chloroplast and the Cd impact on primary photosynthetic processes. Therefore, several alleviative strategies are applied in experimental plant biology to reduce negative Cd effects on plant photosynthesis, growth, and biomass production. Among the recent trends, there are *e.g.*, mitigation of Cd effects by nanoparticles (Kang *et al.* 2024, Soni *et al.* 2024), addition of phosphorus into plant nutrition (Chtouki *et al.* 2021), foliar application of salicylic acid (Hayat *et al.* 2024), and biochar application to soil (Danso *et al.* 2023).

Conclusion: Our study aimed to evaluate the response of PSII to Cd and high light, specifically the activation of protective mechanisms for photosynthetic processes related to photochemical part of photosynthesis, *i.e.*, nonphotochemical quenching and related processes in the chloroplasts. Our study suggests that 10 mM (Cd10) did not cause much negative effects in PSII and its functioning; in contrast, 50 mM (Cd50) induces several negative changes in primary photosynthesis associated with PSII and studied by chlorophyll fluorescence techniques. Results from chlorophyll fluorescence indicate that the most affected site by Cd stress on the electron transport chain is at the plastoquinone level and OEC level, along with the PSII complex. Finally, the combination of Cd stress and high light stress is translated in an enhanced photoinhibitory effect; if the plant is exposed to the heavy metal, a stressor such as high light would have a worse negative effect, and would require more time and energy to recover. Cd-induced changes are, however, not related exclusively to PSII, they comprise a broad complex of responses (for review *see* Bashir *et al.* 2015) including structural changes in thylakoid membrane components, alterations in chloroplast structure, including *e.g.*, reduction in size and number of grana stacks (Hakmaoui *et al.* 2007). In light of the above, our study suggests further research on the various response mechanisms activated by Cd stress and how these mechanisms interact when combined with other stresses, such as high light. Moreover, various plant–microbe interactions in the root compartment of heavy metal-exposed or -treated plants represent an emerging field of study to alleviate negative Cd effects in plants. Recently, several plant growth-promoting bacteria (PGPB) have emerged as promising candidates for enhancing plant resilience to Cd stress. Many PGPB have been tested recently to improve plant tolerance by modulating antioxidant defence mechanisms, facilitating the following aspects of plant physiology: nutrient uptake, enhancing soil quality, regulating plant hormones, photosynthesis, and biomass formation (He *et al.* 2020, Wu *et al.* 2020, Yang *et al.* 2024). Alleviation of negative effects of cadmium toxicity on photosynthetic processes by foliar application of biocompounds is another recent trend (Rafique *et al.* 2025, Shabbir *et al.* 2025) which, together with studies focused on nanoparticles (Faizan *et al.* 2021, Zhou *et al.* 2025), are promising fields of science.

References

Alloway B.J.: Heavy Metals in Soils: Trace Metals and Metalloids in Soils and their Bioavailability. Pp. 614. Springer, Dordrecht 2013.

Baldantoni D., Morra L., Zaccardelli M., Alfani A.: Cadmium accumulation in leaves of leafy vegetables. – Ecotox. Environ. Safe. **123**: 89-94, 2016.

Barták M., Hájek J., Halıcı M.G. *et al.*: Resistance of primary photosynthesis to photoinhibition in Antarctic lichen *Xanthoria elegans*: photoprotective mechanisms activated during a short period of high light stress. – Plants-Basel **12**: 2259, 2023.

Bashir H., Qureshi M.I., Ibrahim M.M., Iqbal M.: Chloroplast and photosystems: impact of cadmium and iron deficiency. – Photosynthetica **53**: 321-335, 2015.

Bednaříková M., Váczi P., Lazár D., Barták M.: Photosynthetic performance of Antarctic lichen *Dermatocarpon polyphyllizum* when affected by desiccation and low temperatures. – Photosynth. Res. **145**: 159-177, 2020.

Bellini E., Maresca V., Betti C. *et al.*: The moss *Leptodictyum riparium* counteracts severe cadmium stress by activation of glutathione transferase and phytochelatin synthase, but slightly by phytochelatins. – Int. J. Mol. Sci. **21**: 1583, 2020.

Ben Ammar W., Mediouni C., Tray B. *et al.*: Glutathione and phytochelatin contents in tomato plants exposed to cadmium. – Biol. Plantarum **52**: 314-320, 2008.

Bharagava R.N., Saxena G.: Bioremediation of Industrial Waste for Environmental Safety. Volume II: Biological Agents and Methods for Industrial Waste Management. Pp. 538. Springer, Singapore 2020.

Bi Y., Chen W., Zhang W. *et al.*: Production of reactive oxygen species, impairment of photosynthetic function and dynamic changes in mitochondria are early events in cadmium-induced cell death in *Arabidopsis thaliana*. – Biol. Cell **101**: 629-643, 2009.

Bussotti F., Kalaji M.H., Desotgiu R. *et al.*: Misurare la vitalità delle piante per mezzo della fluorescenza della clorofilla. [Measuring the vitality of plants using chlorophyll fluorescence.] Pp. 138. Firenze University Press, Florence 2012. [In Italian]

Cai Y., Qi Y., Zhu S.Q. *et al.*: Effects of cadmium stress on photosynthetic apparatus of tobacco. – Appl. Ecol. Env. Res. **21**: 1917-1929, 2023.

Chen X., Tao H., Wu Y., Xu X.: Effects of cadmium on metabolism of photosynthetic pigment and photosynthetic system in *Lactuca sativa* L. revealed by physiological and proteomics analysis. – Sci. Hortic.-Amsterdam **305**: 111371, 2022.

Chen Z., Song S., Wen Y. *et al.*: Toxicity of Cu (II) to the green alga *Chlorella vulgaris*: a perspective of photosynthesis and oxidant stress. – Environ. Sci. Pollut. R. **23**: 17910-17918, 2016.

Cho U.-H., Seo N.-H.: Oxidative stress in *Arabidopsis thaliana* exposed to cadmium is due to hydrogen peroxide accumulation. – Plant. Sci. **168**: 113-120, 2005.

Chtouki M., Naciri R., Soulaimani A. *et al.*: Effect of cadmium and phosphorus interaction on tomato: chlorophyll *a* fluorescence, plant growth, and cadmium translocation. – Water Air Soil Poll. **232**: 84, 2021.

DalCorso G., Farinati S., Furini A.: Regulatory networks of cadmium stress in plants. – Plant Signal. Behav. **5**: 663-667, 2010.

Danso O.P., Acheampong A., Zhang Z. *et al.*: The management of Cd in rice with biochar and selenium: effects, efficiency, and practices. – Carbon Res. **2**: 41, 2023.

Deng G., Li M., Li H. *et al.*: Exposure to cadmium causes declines in growth and photosynthesis in the endangered aquatic fern (*Ceratopteris pteridoides*). – Aquat. Bot. **112**: 23-32, 2014.

Didaran F., Kordrostami M., Ghasemi-Soloklui A.A. *et al.*: The mechanisms of photoinhibition and repair in plants under high light conditions and interplay with abiotic stressors. – J. Photoch. Photobio. B **259**: 113004, 2024.

Dobrikova A.G., Apostolova E.L., Hanć A. *et al.*: Cadmium toxicity in *Salvia sclarea* L.: an integrative response of element uptake, oxidative stress markers, leaf structure and photosynthesis. – Ecotox. Environ. Safe. **209**: 111851, 2021.

El Rasafi T., Oukarroum A., Haddioui A. *et al.*: Cadmium stress in plants: a critical review of the effects, mechanisms, and tolerance strategies. – Crit. Rev. Env. Sci. Tec. **52**: 675-726, 2022.

Faizan M., Alam P., Hussain A. *et al.*: Phytochelatins: key regulator against heavy metal toxicity in plants. – Plant Stress **11**: 100355, 2024.

Faizan M., Bhat J.A., Hessini K. *et al.*: Zinc oxide nanoparticles alleviates the adverse effects of cadmium stress on *Oryza sativa* via modulation of the photosynthesis and antioxidant defense system. – Ecotox. Environ. Safe. **220**: 112401, 2021.

Faller P., Kienzler K., Krieger-Liszkay A.: Mechanism of Cd^{2+} toxicity: Cd^{2+} inhibits photoactivation of Photosystem II by competitive binding to the essential Ca^{2+} site. – BBA-Bioenergetics **1706**: 158-164, 2005.

Faseela P., Sinisha A.K., Brestič M., Puthur J.T.: Chlorophyll *a* fluorescence parameters as indicators of a particular abiotic stress in rice. – Photosynthetica **58**: 293-300, 2020.

Geiken B., Masojídek J., Rizzuto M. *et al.*: Incorporation of [^{35}S] methionine in higher plants reveals that stimulation of the D1 reaction centre II protein turnover accompanies tolerance to heavy metal stress. – Plant Cell Environ. **21**: 1265-1273, 1998.

Genty B., Briantais J.M., Baker N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – BBA-Gen. Subjects **990**: 87-92, 1989.

Gharbi F., Zribi L., Daly A.B. *et al.*: Photosynthetic responses of tomato leaves to salt and cadmium stresses: growth and chlorophyll *a* fluorescence kinetic analyses. – Pol. J. Environ. Stud. **27**: 2499-2508, 2018.

Gonzalez-Mendoza D., Gil F.E., Santamaría J.M., Zapata-Perez O.: Multiple effects of cadmium on the photosynthetic apparatus of *Avicennia germinans* L. as probed by OJIP chlorophyll fluorescence measurements. – Z. Naturforsch. **62c**: 265-272, 2007.

Guo Y., Lu Y., Goltsev V. *et al.*: Comparative effect of tenuazonic acid, diuron, bentazone, dibromothymoquinone and methyl viologen on the kinetics of Chl *a* fluorescence rise OJIP and the MR_{820} signal. – Plant Physiol. Biochem. **156**: 39-48, 2020.

Gururani M.A., Upadhyaya C.P., Strasser R.J. *et al.*: Evaluation of abiotic stress tolerance in transgenic potato plants with reduced expression of PSII manganese stabilizing protein. – Plant Sci. **198**: 7-16, 2013.

Hakmaoui A., Ater M., Bóka K., Barón M.: Copper and cadmium tolerance, uptake and effect on chloroplast ultrastructure. Studies on *Salix purpurea* and *Phragmites australis*. – Z. Naturforsch. **62c**: 417-426, 2007.

Han Z., Wei X., Wan D. *et al.*: Effect of molybdenum on plant physiology and cadmium uptake and translocation in rape (*Brassica napus* L.) under different levels of cadmium stress. – Int. J. Env. Res. Pub. He. **17**: 2355, 2020.

Haq S.I.U., Benita M.B., de Caralt S.: Photoinhibition and recovery of primary photosynthesis in Antarctic and subantarctic lichens. Analysis of interspecific differences. – Czech Polar Rep. **14**: 51-76, 2024.

Hayat U., din K.u., Haider A. *et al.*: Salicylic acid-induced antioxidant defense system alleviates cadmium toxicity in wheat. – J. Soil Sci. Plant Nutr. **24**: 3068-3086, 2024.

He L., Yue Z., Chen C. *et al.*: Enhancing iron uptake and alleviating iron toxicity in wheat by plant growth-promoting bacteria: theories and practices. – Int. J. Agric. Biol. **23**: 190-196, 2020.

Hernandez H.P.V.: Effects of heavy metals ions on primary photosynthetic processes in Antarctic filamentous alga *Zygnema* sp. – Czech Polar Rep. **6**: 180-185, 2016.

Hikosaka K.: Photosynthesis, chlorophyll fluorescence and photochemical reflectance index in photoinhibited leaves. – Funct. Plant Biol. **48**: 815-826, 2021.

Huang X., Chen H., Chen H. *et al.*: Spatiotemporal heterogeneity of chlorophyll content and fluorescence response within rice (*Oryza sativa* L.) canopies under different cadmium stress. – Agronomy **13**: 121, 2023.

Jiang C.-D., Jiang G.-M., Wang X. *et al.*: Increased photosynthetic activities and thermostability of photosystem II with leaf development of elm seedlings (*Ulmus pumila*) probed by the fast fluorescence rise OJIP. – Environ. Exp. Bot. **58**: 261-268, 2006.

Jin H., Liu B., Luo L. *et al.*: HYPERSENSITIVE TO HIGH LIGHT1 interacts with LOW QUANTUM YIELD OF PHOTOSYSTEM II1 and functions in protection of photosystem II from photodamage in *Arabidopsis*. – Plant Cell **26**: 1213-1229, 2014.

Johnson M.P., Ruban A.V.: Restoration of rapidly reversible photoprotective energy dissipation in the absence of PsbS protein by enhanced ΔpH. – J. Biol. Chem. **286**: 19973-19981, 2011.

Johnson M.P., Zia A., Ruban A.V.: Elevated ΔpH restores rapidly reversible photoprotective energy dissipation in *Arabidopsis* chloroplasts deficient in lutein and xanthophyll cycle activity. – Planta **235**: 193-204, 2012.

Kalaji H.M., Jajoo A., Oukarroum A. *et al.*: The use of chlorophyll fluorescence kinetics analysis to study the performance of photosynthetic machinery in plants. – In: Ahmad P., Rasool S. (ed.): Emerging Technologies and Management of Crop Stress Tolerance: A Sustainable Approach. Pp. 347-384. Academic Press, San Diego 2014.

Kalaji H.M., Loboda T.: Photosystem II of barley seedlings under cadmium and lead stress. – Plant Soil Environ. **53**: 511-516, 2007.

Kang Y., Qin H., Wang G. *et al.*: Selenium nanoparticles mitigate cadmium stress in tomato through enhanced accumulation and transport of sulfate/selenite and polyamines. – J. Agr. Food Chem. **72**: 1473-1486, 2024.

Krall J.P., Edwards G.E.: Relationship between photosystem II activity and CO_2 fixation in leaves. – Physiol. Plantarum **86**: 180-187, 1992.

Kramer D.M., Johnson G., Kiirats O., Edwards G.E.: New fluorescence parameters for the determination of Q_A redox state and excitation energy fluxes. – Photosynth. Res. **79**: 209-218, 2004.

Krüger G.H.J., De Villiers M.F., Strauss A.J. *et al.*: Inhibition of photosystem II activities in soybean (*Glycine max*) genotypes differing in chilling sensitivity. – S. Afr. J. Bot. **95**: 85-96, 2014.

Küpper H., Parameswaran A., Leitenmaier B. *et al.*: Cadmium-induced inhibition of photosynthesis and long-term acclimation to cadmium stress in the hyperaccumulator *Thlaspi caerulescens*. – New Phytol. **175**: 655-674, 2007.

Li J., Yi C., Zhang C. *et al.*: Effects of light quality on leaf growth and photosynthetic fluorescence of *Brasenia schreberi* seedlings. – Heliyon **7**: e06082, 2021.

Li X.-P., Björkman O., Shih C. *et al.*: A pigment-binding protein essential for regulation of photosynthetic light harvesting. – Nature **403**: 391-395, 2000.

Lichtenthaler H.K., Buschmann C., Knapp M.: How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio R_{Fd} of leaves with the PAM fluorometer. – Photosynthetica **43**: 379-393, 2005.

Lin Y.-F., Aarts M.G.M.: The molecular mechanism of zinc and cadmium stress response in plants. – Cell. Mol. Life Sci. **69**: 3187-3206, 2012.

Linger P., Ostwald A., Haensler J.: *Cannabis sativa* L. growing on heavy metal contaminated soil: growth, cadmium uptake and photosynthesis. – Biol. Plantarum **49**: 567-576, 2005.

Liu L., Li W., Song W., Guo M.: Remediation techniques for heavy metal-contaminated soils: principles and applicability. – Sci. Total Environ. **633**: 206-219, 2018.

Maksymiec W., Wójcik M., Krupa Z.: Variation in oxidative stress and photochemical activity in *Arabidopsis thaliana* leaves subjected to cadmium and excess copper in the presence or absence of jasmonate and ascorbate. – Chemosphere **66**: 421-427, 2007.

Malnoë A.: Photoinhibition or photoprotection of photosynthesis? Update on the (newly termed) sustained quenching component qH. – Environ. Exp. Bot. **154**: 123-133, 2018.

Manzoor H., Mehwish, Bukhat S. *et al.*: Methyl jasmonate alleviated the adverse effects of cadmium stress in pea (*Pisum sativum* L.): a nexus of Photosystem II activity and dynamics of redox balance. – Front. Plant Sci. **13**: 860664, 2022.

Martínez-Peñalver A., Graña E., Reigosa M.J., Sánchez-Moreiras A.M.: The early response of *Arabidopsis thaliana* to cadmium- and copper-induced stress. – Environ. Exp. Bot. **78**: 1-9, 2012.

Maslać A., Maslać M., Tkalec M.: The impact of cadmium on photosynthetic performance and secondary metabolites in the lichens *Parmelia sulcata*, *Flavoparmelia caperata* and *Evernia prunastri*. – Acta Bot. Croat. **75**: 186-193, 2016.

Matsuoka T., Onozawa A., Sonoike K., Kore-eda S.: Crassulacean acid metabolism induction in *Mesembryanthemum crystallinum* can be estimated by non-photochemical quenching upon actinic illumination during the dark period. – Plant Cell Physiol. **59**: 1966-1975, 2018.

Merlos Rodrigo M.A., Anjum N.A., Heger Z. *et al.*: Role of phytochelatins in redox caused stress in plants and animals. – In: Shanker A.K., Shanker C. (ed.): Abiotic and Biotic Stress in Plants: Recent Advances and Future Perspectives. InTech, London 2016.

Mou R.X., Cao Z.Y., Lin X.Y. *et al.*: Characterization of the phytochelatins and their derivatives in rice exposed to cadmium based on high-performance liquid chromatography coupled with data-dependent hybrid linear ion trap orbitrap mass spectrometry. – Rapid Commun. Mass Spectrom. **30**: 1891-1900, 2016.

Moustakas M., Dobrikova A., Sperdouli I. *et al.*: Photosystem II tolerance to excess zinc exposure and high light stress in *Salvia sclarea* L. – Agronomy **14**: 589, 2024.

Moustakas M., Hanć A., Dobrikova A. *et al.*: Spatial heterogeneity of cadmium effects on *Salvia sclarea* leaves revealed by chlorophyll fluorescence imaging analysis and laser ablation inductively coupled plasma mass spectrometry. – Materials **12**: 2953, 2019.

Naciri R., Chtouki M., Oukarroum A. *et al.*: Mechanisms of cadmium mitigation in tomato plants under orthophosphate and polyphosphate fertilization regimes. – Ecotox. Environ. Safe. **274**: 116219, 2024.

Nishijo M., Nakagawa H., Suwazono Y. *et al.*: Causes of death in patients with Itai-itai disease suffering from severe chronic cadmium poisoning: a nested case – control analysis of a follow-up study in Japan. – BMJ Open **7**: e015694, 2017.

Orekhova A., Barták M., Casanova-Katny A., Hájek J.: Resistance of Antarctic moss *Sanionia uncinata* to photoinhibition: chlorophyll fluorescence analysis of samples from the western and eastern coasts of the Antarctic Peninsula. – Plant Biol. **23**: 653-663, 2021.

Pagliano C., Raviolo M., Dalla Vecchia F. *et al.*: Evidence for PSII donor-side damage and photoinhibition induced by cadmium treatment on rice (*Oryza sativa* L.). – J. Photoch. Photobio. B **84**: 70-78, 2006.

Parmar P., Kumari N., Sharma V.: Structural and functional alterations in photosynthetic apparatus of plants under cadmium stress. – Bot. Stud. **54**: 45, 2013.

Peng J.-S., Gong J.-M.: Vacuolar sequestration capacity and long-distance metal transport in plants. – Front. Plant Sci. **5**: 19, 2014.

Piotto F.A., Carvalho M.E.A., Souza L.A. *et al.*: Estimating tomato tolerance to heavy metal toxicity: cadmium as study case. – Environ. Sci. Pollut. R. **25**: 27535-27544, 2018.

Pogson B.J., Niyogi K.K., Björkman O., DellaPenna D.: Altered xanthophyll compositions adversely affect chlorophyll accumulation and nonphotochemical quenching in *Arabidopsis* mutants. – PNAS **95**: 13324-13329, 1998.

Radeva V., Petrov V., Minkov I. *et al.*: Effect of cadmium on *Arabidopsis thaliana* mutants tolerant to oxidative stress. – Biotechnol. Biotec. Eq. **24**: 113-118, 2010.

Rafique M., Noreen Z., Usman S. *et al.* Mitigation of adverse effect of cadmium toxicity in lettuce (*Lactuca sativa* L.) through foliar application of chitosan and spermidine. – Sci.

Rep.-UK **15**: 9062, 2025.
Roháček K.: Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. – Photosynthetica **40**: 13-29, 2002.
Shabbir A., Shah A.A., Usman S. *et al.*: Efficacy of malic and tartaric acid in mitigation of cadmium stress in *Spinacia oleracea* L. *via* modulations in physiological and biochemical attributes. – Sci. Rep.-UK **15**: 3366, 2025.
Sharma A., Kumar V., Shahzad B. *et al.*: Photosynthetic response of plants under different abiotic stresses: a review. – J. Plant Growth Regul. **39**: 509-531, 2020.
Sharma N., Nagar S., Thakur M. *et al.*: Photosystems under high light stress: throwing light on mechanism and adaptation. – Photosynthetica **61**: 250-263, 2023.
Shay P.-E., Kubien D.S.: Field analysis of photoprotection in co-occurring cool climate C_3 and C_4 grasses. – Physiol. Plantarum **147**: 316-328, 2013.
Singh H., Kumar D., Soni V.: Performance of chlorophyll *a* fluorescence parameters in *Lemna minor* under heavy metal stress induced by various concentration of copper. – Sci. Rep.-UK **12**: 10620, 2022.
Singh H., Kumar D., Soni V.: Impact of mercury on photosynthetic performance of *Lemna minor*: a chlorophyll fluorescence analysis. – Sci. Rep.-UK **13**: 12181, 2023.
Song Y., Jin L., Wang X.: Cadmium absorption and transportation pathways in plants. – Int. J. Phytoremediat. **19**: 133-141, 2017.
Soni S., Jha A.B., Dubey R.S., Sharma P.: Mitigating cadmium accumulation and toxicity in plants: the promising role of nanoparticles. – Sci. Total Environ. **912**: 168826, 2024.
Strasser R.J., Tsimilli-Michael M., Srivastava A.: Analysis of the chlorophyll *a* fluorescence transient. – In: Papageorgiou G.C., Govindjee (ed.): Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Advances in Photosynthesis and Respiration. Pp. 321-362. Springer, Dordrecht 2004.
Szopiński M., Sitko K., Gieroń Ż. *et al.*: Toxic effects of Cd and Zn on the photosynthetic apparatus of the *Arabidopsis halleri* and *Arabidopsis arenosa* pseudo-metallophytes. – Front. Plant Sci. **10**: 748, 2019.
Tietz S., Hall C.C., Cruz J.A., Kramer D.M.: $NPQ_{(T)}$: a chlorophyll fluorescence parameter for rapid estimation and imaging of non-photochemical quenching of excitons in photosystem-II-associated antenna complexes. – Plant Cell Environ. **40**: 1243-1255, 2017.
Todorenko D., Volgusheva A., Timofeev N. *et al.*: Multiple *in vivo* effects of cadmium on photosynthetic electron transport in pea plants. – Photochem. Photobiol. **97**: 1516-1526, 2021.
Tomar R.S., Jajoo A.: Photosynthetic response in wheat plants caused by the phototoxicity of fluoranthene. – Funct. Plant Biol. **46**: 725-731, 2019.
Van Belleghem F., Cuypers A., Semane B. *et al.*: Subcellular localization of cadmium in roots and leaves of *Arabidopsis thaliana*. – New Phytol. **173**: 495-508, 2007.
Vanhoudt N., Horemans N., Biermans G. *et al.*: Uranium affects photosynthetic parameters in *Arabidopsis thaliana*. – Environ. Exp. Bot. **97**: 22-29, 2014.
Verma N., Prasad S.M.: Interplay of hydrogen peroxide and nitric oxide: systemic regulation of photosynthetic performance and nitrogen metabolism in cadmium challenged cyanobacteria. – Physiol. Mol. Biol. Pla. **27**: 2181-2199, 2021.
Waheed A., Zhang Q., Xu H. *et al.*: Mitigation of cadmium stress by salicylic acid: physiological and biochemical responses in NM-2006, NM-92, and Mash-88 mung bean varieties. – J. Hazard. Mater. **485**: 136878, 2025.
Wang C., Gu Q., Zhao L. *et al.*: Photochemical efficiency of photosystem II in inverted leaves of soybean [*Glycine max* (L.) Merr.] affected by elevated temperature and high light. – Front. Plant Sci. **12**: 772644, 2022c.
Wang H., Wang X.-Q., Zeng Z.-L. *et al.*: Photosynthesis under fluctuating light in the CAM plant *Vanilla planifolia*. – Plant Sci. **317**: 111207, 2022e.
Wang Q., Xie D., Peng L. *et al.*: Phytotoxicity of atrazine combined with cadmium on photosynthetic apparatus of the emergent plant species *Iris pseudacorus*. – Environ. Sci. Pollut. R. **29**: 34798-34812, 2022b.
Wang S., Duo J., Wufuer R. *et al.*: The binding ability of mercury (Hg) to Photosystem I and II explained the difference in its toxicity on the two photosystems of *Chlorella pyrenoidosa*. – Toxics **10**: 455, 2022a.
Wang S., Wufuer R., Duo J. *et al.*: Cadmium caused different toxicity to Photosystem I and Photosystem II of freshwater unicellular algae *Chlorella pyrenoidosa* (Chlorophyta). – Toxics **10**: 352, 2022d.
Wei J., Huang H., Zhang S. *et al.*: Functions of violaxanthin de-epoxidase-related (VDR) in the photoprotective response to high-light stress. – Plant Growth Regul. **104**: 187-200, 2024.
Wen X., Qiu N., Lu Q., Lu C.: Enhanced thermotolerance of photosystem II in salt-adapted plants of the halophyte *Artemisia anethifolia*. – Planta **220**: 486-497, 2005.
White A.J., Critchley C.: Rapid light curves: a new fluorescence method to assess the state of the photosynthetic apparatus. – Photosynth. Res. **59**: 63-72, 1999.
Wodala B., Eitel G., Gyula T.N. *et al.*: Monitoring moderate Cu and Cd toxicity by chlorophyll fluorescence and P700 absorbance in pea leaves. – Photosynthetica **50**: 380-386, 2012.
Wu Y., Ma L., Liu Q. *et al.*: The plant-growth promoting bacteria promote cadmium uptake by inducing a hormonal crosstalk and lateral root formation in a hyperaccumulator plant *Sedum alfredii*. – J. Hazard. Mater. **395**: 122661, 2020.
Xin J., Zhao X.H., Tan Q.L. *et al.*: Effects of cadmium exposure on the growth, photosynthesis, and antioxidant defense system in two radish (*Raphanus sativus* L.) cultivars. – Photosynthetica **57**: 967-973, 2019.
Yang X., Li J., Yang Z. *et al.*: Plant growth promoting bacteria and citric acid promote growth and cadmium phytoremediation in ryegrass. – Int. J. Phytoremediat. **26**: 382-392, 2024.
Zhao H., Wang W., Fan Y. *et al.*: Physiological, photosynthetic characteristic and transcriptome analysis of *PsnWRKY70* transgenic *Populus simonii* × *Populus nigra* under salt stress. – Int. J. Mol. Sci. **26**: 81, 2025.
Zhou L., Zhou L., Wu H. *et al.*: Application of chlorophyll fluorescence analysis technique in studying the response of lettuce (*Lactuca sativa* L.) to cadmium stress. – Sensors **24**: 1501, 2024a.
Zhou R., Xu J., Li L. *et al.*: Exploration of the effects of cadmium stress on photosynthesis in *Oenanthe javanica* (Blume) DC. – Toxics **12**: 307, 2024b.
Zhou W., Juneau P., Qiu B.: Growth and photosynthetic responses of the bloom-forming cyanobacterium *Microcystis aeruginosa* to elevated levels of cadmium. – Chemosphere **65**: 1738-1746, 2006.
Zhou X., El-Sappah A.H., Khaskhoussi A. *et al.*: Nanoparticles: a promising tool against environmental stress in plants. – Front. Plant Sci. **15**: 1509047, 2025.
Zsiros O., Nagy G., Patai R. *et al.*: Similarities and differences in the effects of toxic concentrations of cadmium and chromium on the structure and functions of thylakoid membranes in *Chlorella variabilis*. – Front. Plant Sci. **11**: 1006, 2020.