# Does Cd possess the stimulatory effect on gas exchange and photochemistry in C3-CAM intermediate plants?

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**Research Article**

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# Abstract

The soil-grown semi-halophytic CAM (crassulacean acid metabolism) facultative model plant - *Mesembryanthemum crystallinum* L. (common ice plant) exhibits minute toxicity symptoms when exposed to elevated cadmium doses. In this study, photochemical activity as well as gas exchange of the soil-grown $C_3$-CAM- performing plants to increased cadmium concentrations (0.01-10.0 mM) were investigated. An increase of net photosynthesis ($P_N$) observed in $C_3$- performing plants upon exposure to Cd runs in parallel with the rise of transpiration level. As the transpiration process tends to be elevated also in darkness, it gives rise to the suggestion that the first effect of Cd presence is stimulation of stomata aperture. Also, photochemical activity is well-secured in Cd-treated plants, which implies the involvement of additional mechanisms triggered to protect *M. crystallinum* against Cd toxicity.


# 1. Introduction

Some heavy metals (HMs), when applied at the appropriate level, are required for plant development. However, some others, such as cadmium, belong to the group of the most toxic HMs that are biologically non-essential and may cause toxicity symptoms in plants even in small amounts (Chibuike and Obiora 2014). Interestingly, it has been reported about Cd-carbonic anhydrase described in diatoms, indicating that those organisms may use Cd for their activity (Lane et al. 2005). Cadmium toxicity brings about a wide range of damages on molecular, cellular, and organ levels (Gallego et al. 2011), of which the most known symptoms are chlorotic and necrotic changes in shoot and root structures as well as the biomass decrease. These manifestations result from alterations in mineral nutrition, carbohydrate metabolism, and water management (Nazar et al. 2012; Mariem et al. 2014; Ismael et al. 2019). In particular, when the photosynthetic apparatus is exposed to Cd, such parameters as the net photosynthesis rate, photochemical activity, stomatal conductance, and transpiration may be considerably inhibited (Gallego et al. 2011; Moradi and Ehsanzadeh 2015). In response to the Cd presence in the environment, plants carry out different defense mechanisms such as immobilization of toxic ions, mainly by their sequestration in the vacuoles, increased nitrogen and sulfur uptakes accompanied by manganese and zinc accumulation, synthesis of metallothioneins, phytochelatins, stress proteins and increased expression of antioxidant enzymes (Carvalho et al. 2019). There are also some other mechanisms including an increase of non-photochemical quenching and cyclic electron transport in PSI as well as the increase of phenolic and anthocyanins that may protect photosynthetic apparatus against Cd toxicity. This phenomenon is clearly visible in *Salvia sclarea* plants under Cd treatment (Moustakas et al. 2019; Dobrikova et al. 2021).

Halophytes, collectively referring to salinity-tolerant plants, were proposed as useful models for phytoremediation since their salt tolerance may play a key role in cases of Cd contamination, where both the metal presence and high salinity often act as concurrent physiological stressors (Manousaki and Kalogerakis 2011). Furthermore, halophytes can often tolerate unfavorable environmental factors such as drought, heat, and frost (Deinlein et al. 2014). The common ice plant (*Mesembryanthemum crystallinum* L.) occurs naturally in habitats affected by an excess of different toxic ions. This plant is

often used as a model in physiological and molecular research due to its ability for a reversible photosynthetic metabolism switch from $C_3$ to CAM (Crassulacean acid metabolism) (Cushman and Borland 2002; Nosek et al. 2018, 2021). As a $C_3$-performing plant, it can fix $CO_2$ during the day, when stomata are open, while in the CAM mode the stomata remain closed during the day and the process of $CO_2$ fixation occurs mostly at night (Cushman and Borland 2002). Our recent studies confirmed that *M. crystallinum* can accumulate relatively large amounts of Cd without visible toxicity symptoms (Nosek et al. 2019, 2020). However, the impact of the Cd ion was not tested yet in this facultative CAM semi-halophyte in the context of the primary metabolic process: photosynthesis rate and transpiration.

This research aimed to determine, how the photosynthesis and respiration of either $C_3$- and CAM-performing *M. crystallinum* are affected by exposure to high Cd concentrations. We also attempted to reveal whether alterations of gas exchange or photochemical activity of leaves exposed to Cd treatment were primary or secondary effects.

# 2. Material And Methods

## 2.1. Growth of plants and Cd treatment

The seeds of *M. crystallinum* originated from the Botanical Garden of the Technical University in Darmstadt (Germany). They were sown in pots filled with a 1:4 (v/v) mix of sand and garden soil ("Athena" Bio-Products, Golczewo, Poland; pH 6.75; d = 0.24 kg $dm^{-3}$). After 2 weeks the seedlings were planted in 9 x 9 x 10 cm pots. The cultivation was carried out according to Nosek et al. (2020) in the summer season in a greenhouse with a photoperiod 16/8 h (day/night; 25ºC/17ºC) and white light intensity 300–350 µmol $m^{-2}$ $s^{-1}$ PAR, relative humidity 65–70%. Six weeks after sowing, when fifth whorls of leaves were formed, one group of plants was watered with 0.4 M NaCl solution to induce CAM photosynthesis, while the second group was watered with tap water ($C_3$ plants). After 14 days, CAM was confirmed with the measurement of the diurnal Δ-malate (the difference between malate concentration measured at the beginning and the end of the light phase) in the leaf cell sap according to the protocol described earlier (Gawronska and Niewiadomska 2015). Next, each metabolic group ($C_3$ or CAM) was divided into 5 variants. Four of the plant variants were treated with different concentrations of $CdCl_2$ solution: 0.01 mM, 0.1 mM, 1.0 mM, and 10.0 mM for 8 days, and the remaining set of plants served as a control (irrigated with water).

## 2.2. Measurements of chlorophyll a fluorescence

The efficiency of PSII was assessed by the measurement of the rate of fluorescence quenching at room temperature using a Handy-PEA fluorimeter (*Hansatech Instruments, UK*). The measurements were carried out on the day following the last application of the $CdCl_2$ solution; saturation light was 3500 µmol $m^{-2}$ $s^{-1}$. After the plants had been dark-adapted for 20 minutes, pulsed light was emitted to induce fluorescence. The following parameters of chlorophyll fluorescence were calculated: maximum PSII quantum yield (Fv/Fm), maximum efficiency of water splitting complex on the PSII side (Fv/Fo),

performance index calculated based on energy absorption ($PI_{ABS}$), the flow of retained photons (reducing $Q_A$) in PSII per reaction center (RC) ($TR_0$/RC), electron transport through carriers located in the electron transport chain after $Q_A$ per RC ($ET_0$/RC) and the ratio of the total dissipation of untrapped excitation energy from all RCs to the number of active RCs ($DI_0$/RC) (Strasser et al. 2000).

## 2.3. Gas exchange measurements

Gas exchange of control plants and plants treated with different Cd concentrations was monitored using a LCpro-SD gas exchange instrument (*ADC Bioscentific Ltd, UK*) with a 6.24 $cm^2$ cuvette equipped with a head with mix red/blue LED light source. The measurements were made at high $CO_2$ concentration (~ 400 $\mu mol \cdot mol^{-1}$), 300 $\mu mol\ s^{-1}$ of airflow, RH 50–55%, leaf temperature 28ºC, and the red light intensity equal to 130 $\mu mol\ m^{-2}\ s^{-1}$. Gas exchange measurements were carried out on at least four plants of each variant of the applied Cd concentration. Net photosynthesis was depicted as the rate of carbon fixation during photosynthesis *minus* the rate of carbon dioxide simultaneously lost during respiration (Tokarz et al. 2019).

## 2.4. Statistical analyses

The results were analysed with Statistica 13.3 (StatSoft, USA) statistical software. One-way ANOVA followed by post-hoc test was used to evaluate individual treatment effects at $P \leq 0.05$.

# 3. Results

## 3.1. Gas exchange in $C_3$ and CAM- performing plants under Cd treatment

Transpiration of $C_3$-performing plants treated with Cd at 0.01-1.0 mM concentrations increased when measured both in the light and in the dark (Fig. 1a, 1b). In CAM-performing plants, none of the applied Cd concentrations had significant effect on plant transpiration. (Fig. 1a). In the dark, Cd applied at higher concentrations stimulated transpiration (Fig. 1b). Overall, in the light as well as in the dark the transpiration rate was significantly higher in $C_3$- than in CAM-performing plants. An increase of $P_N$ in plants of both metabolic states was observed (Fig. 1c). In $C_3$-performing plants treated with different Cd concentrations a decrease in the respiration process was observed which correlated with a $P_N$ increase (Fig. 1d). In CAM plants under Cd treatment (with an exception of 10.0 mM) an increase of respiration intensity was noted.

## 3.2. Photochemical activity in $C_3$ and CAM-performing plants under Cd treatment

The values of Fv/Fm did not change significantly upon Cd treatment for both metabolic groups (Fig. 2a). In addition, there were no statistical differences between plants treated with different variants of $CdCl_2$ in comparison to control plants (with one exception for 0.01 mM Cd). In both metabolic groups, Cd did not alter the $PI_{ABS}$ parameter (the exception for 0.01 mM Cd, where an increase in $PI_{ABS}$ values was shown in $C_3$-performing plants) (Fig. 2b). In $C_3$-performing plants treated with 0.1 mM $CdCl_2$ the highest values of Fv/Fo were observed, while in CAM plants Cd did not affect the mentioned parameter (Fig. 2c). The dissipated energy flux per active reaction center ($DI_0$/RC) did not change in $C_3$ plants (Fig. 2d) while in CAM mode the values of this parameter decreased in plants treated with 0.01–1.0 mM Cd. Statistically similar values of $ET_0$/RC obtained for both $C_3$ and CAM plants (Fig. 2e) prove that Cd application did not significantly alter the function of this part of the electron transport chain. In $C_3$ plants, about 1.5-fold higher values were observed compared to CAM. In $C_3$-performing plants, Cd did not affect $TR_0$/RC (Fig. 2f), whereas, in CAM mode, Cd applied at a concentration of 0.01 and 0.1 mM caused the decline of these values. In all the analyzed variants of Cd treatment about 1.5-fold higher values were observed for $C_3$ as compared to CAM plants. Detailed results regarding chlorophyll *a* fluorescence induction curve in leaves of $C_3$- and CAM-performing plants under Cd treatment are presented in the Online Resource.

# 4. Discussion

Plant exposure to cadmium affects the primary metabolism of plants, also photosynthesis in different ways: by inhibition of chlorophyll synthesis, alterations in lipoprotein-chlorophyll complexes, and an increase of lipid peroxidation (Moradi and Ehsanzadeh 2015). Chlorophyll *a* fluorescence, as well as gas exchange, are two of the relatively fast and easy methods allowing the assessment of Cd impact on plants (Dias et al. 2013). It was previously shown that in *Brassica juncea* the treatment with 25.0 μM Cd impacted gas exchange leading to a decrease in the transpiration process (Haag-Kerwer et al. 1999; Prasad et al. 2001). In *Triticum aestivum* treated with 100 μM of Cd for 28 days, a decrease in $P_N$ and transpiration was observed (Arshad et al. 2015). However, Cd at lower concentrations caused an increase in $P_N$ in a hyperaccumulator *Lonicera japonica* (Jia et al. 2015). Plants exposed to the so-called slight metal stress may exhibit enhanced respiration intensity (Lösch et al. 1999) as shown in *Lemma trisulca* upon treatment with 5.0 mM Cd (Prasad et al. 2001). The authors suggested that the stress caused by Cd could mobilize plant metabolism and consequently lead to an increase in respiration.

This stimulatory effect of low doses of heavy metals (such as Cd) called hormesis has been recently widely investigated in plants (Adamakis et al. 2020; Moustakas et al. 2022). Some mechanisms such as an increase in the level of auxin and flavonol as well as upkeep of similar content of hydrogen peroxide in control and tested plants have been reported to induce a better performance of plants exposed to Cd treatment (Carvalho et al. 2020; Małkowski et al. 2020). Number of studies have also reported the stimulatory effect of Cd to PSII observed as an increase in non-photochemical quenching to protect photosynthetic apparatus by inhibiting excessive reactive oxygen species production. It is considered that hormesis may be evolutionary adaptation as the response to environmental stress factors (Moustakas et al. 2022).

In our previous studies regarding the influence of Cd toxicity on soil-grown CAM facultative and halophyte model plant – *M. crystallinum*, none of the tested biometric parameters suggested a malignant effect of applied Cd ions (Nosek et al. 2019). Different abiotic stressors i.e. drought, high light, and elevated salinity may induce a shift towards CAM performance (Winter 2019). Encouraged by that fact, in our previous study we tested if a shift towards CAM photosynthesis performance may be initiated with elevated Cd concentrations as well. The Δ-malate (difference in malate concentration between the beginning and end of the light phase of photoperiod), as well as $^{13}C$ discrimination analysis both of which are the hallmarks of functional CAM, revealed no remarks of CAM presence in Cd-treated $C_3$-performing plants, confirming that Cd does not belong to the group of abiotic stress factors capable of inducing CAM photosynthesis (Nosek et al. 2019).

To solve the discrepancies found in the above-cited literature we attempted to determine if Cd might impact gas exchange in *M. crystallinum* plants performing $C_3$ or CAM metabolism in light and darkness. We intended to answer what is the primary effect of Cd as observed in our plant material. Based on the results presented in Fig. 1a and 1c we can suspect that the stimulation of $P_N$ runs in parallel to increased transpiration (at least in the range of 0.1–1.0 mM Cd). As this result could also be observed in the dark (Fig. 2b) it seems that the first effect is stimulation of stomata aperture. For CAM plants, the stimulatory effect of Cd is not observed as stomata are mostly closed during the day phase. During the night Cd can stimulate respiration only in CAM-performing plants whereas in the $C_3$ phase the opposite process takes place. The lack of damage within the photosynthetic apparatus upon Cd treatment (Fig. 1) is confirmed by the results of the photochemical activity. In CAM plants, a low level of transpiration is associated with stomata closure during the day. In this metabolic group, due to the photosynthetic metabolism, the $P_N$ values are much lower compared to the $C_3$-performing plants. The analysis of $P_N$ values indicates that Cd may cause an increase in $CO_2$ fixation rate at lower Cd concentrations and thus improve photosynthesis. A decrease in respiration intensity noted in our experiments may be related to the accumulated Cd portion which was shown in our previous research (Nosek et al. 2019).

The Fv/Fm parameter is usually used as a stress indicator in the assessment of plants' response to various stress factors. The average potential performance of PSII is assumed to be not affected if values of the Fv/Fm parameter are higher than 0.8 (Björkman and Demmig 1987). Our experiments indicated high plant tolerance to Cd presence and lack of symptoms of oxidative stress. Also, in both metabolic states, no significant changes in Fv/Fo and $PI_{ABS}$ parameters were observed. Similarly, the $PI_{ABS}$ parameter together with relative ETR was not altered in *Medicago truncatula* plants under Cd treatment. However, this was observed only in mycorrhizal plants suggesting that mycorrhizal may positively influence photochemical activity (Aloui et al. 2011). Also, the rhizospheric bacteria may increase plants tolerance towards Cd (Supel et al. 2022). Cd was shown to reduce electron flow through PSII and enhance non-photochemical quenching (Larsson et al. 1998). In our experiments (Fig. 2d) the $DI_O$/RC parameter was not altered by Cd in $C_3$-performing plants. However, in CAM plants a decrease in these values was observed only in plants treated with Cd at concentrations 0.01–1.0 mM. This can occur due to a tendency to increase the $P_N$ values (Fig. 1c) and to lower the dissipation of excess energy. In our research, no

negative Cd impact on electron transport from plastoquinone $Q_A$ ($ET_0$/RC) was observed and the differences between $C_3$- and CAM plants resulted from osmotic stress presence in CAM-performing plants, as in this metabolic group reduced participation in PSII in the overall photosynthesis process can be expected. These results confirm our previous study, where osmotic stress-induced CAM photosynthesis was responsible for the downregulation of photosynthetic apparatus efficiency (Nosek et al. 2022). In our research, the lack of alterations in $C_3$- and CAM-performing plants, also at the highest applied treatment (10.0 mM Cd) may result from the occurrence of an additional mechanism that may protect plants from Cd toxicity.

# 5. Conclusions

Changes in transpiration under Cd treatment may result from stomata opening which subsequently leads to altered photosynthesis intensity, especially in $C_3$-performing plants. The applied Cd doses do not affect Fv/Fm indicating that the oxidative stress is rather unlikely. An increase in $P_N$ values is correlated with decreased absorbed energy dissipation (measured as $DI_0$/RC). Analogous changes are not observed in CAM plants because of different metabolic performances.

High plant tolerance to even the highest Cd concentration (10.0 mM) indicates the presence of additional, yet unrevealed mechanisms that can protect the photosynthetic apparatus against Cd negative effects. Due to the high resistance of *M. crystallinum* to Cd toxicity, this plant may be useful in the phytoremediation of polluted areas.

# Declarations

### Ethical Approval

Not applicable

### Consent to Participate

Not applicable

### Consent to Publish

Not applicable

### Author Contributions

Zbigniew Miszalski designed this work. Adriana Kaczmarczyk, Paweł Kaszycki, Paulina Supel and Zbigniew Miszalski provided study materials, performed the experiments and analyzed the results. Adriana Kaczmarczyk and Michał Nosek applied statistical techniques to analyze study data. Adriana Kaczmarczyk drafted the manuscript and Michał Nosek, Paweł Kaszycki and Zbigniew Miszalski revised it. All authors have read and approved the final manuscript.

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### Competing Interests

The authors have no competing interests to declare that are relevant to the content of this article.

### Availability of data and materials

The dataset used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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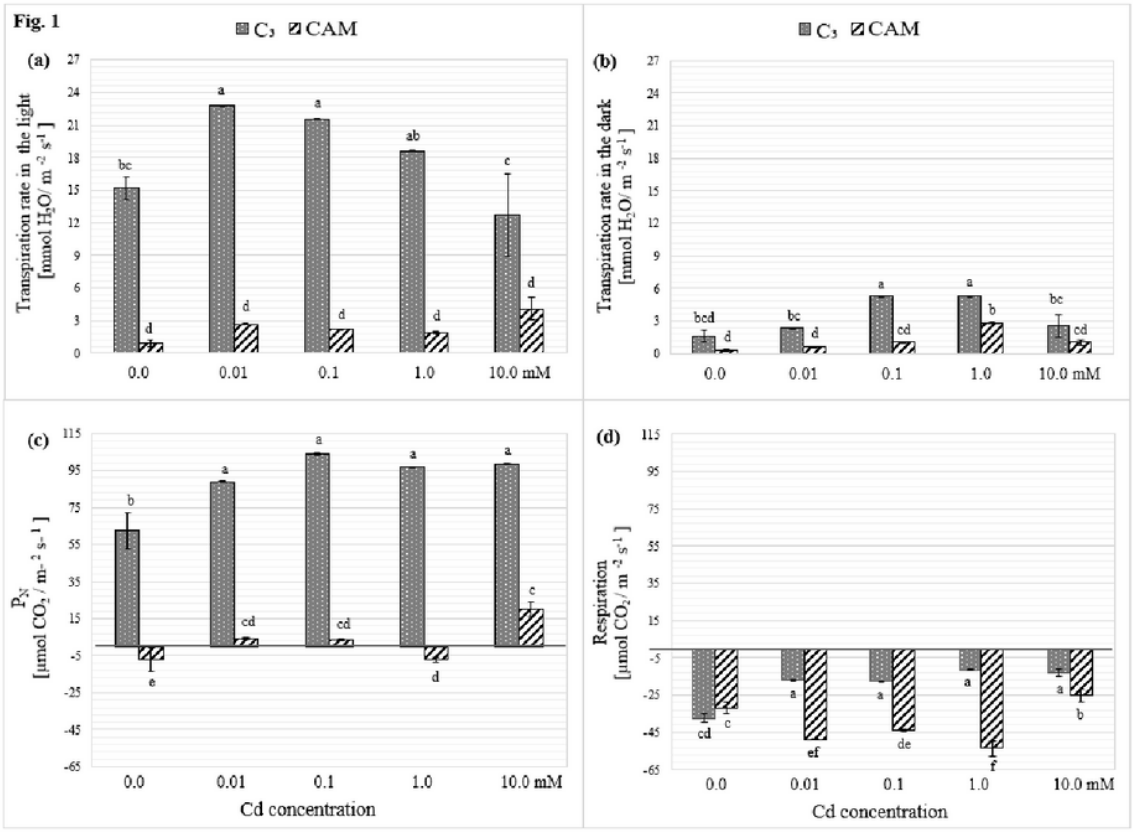
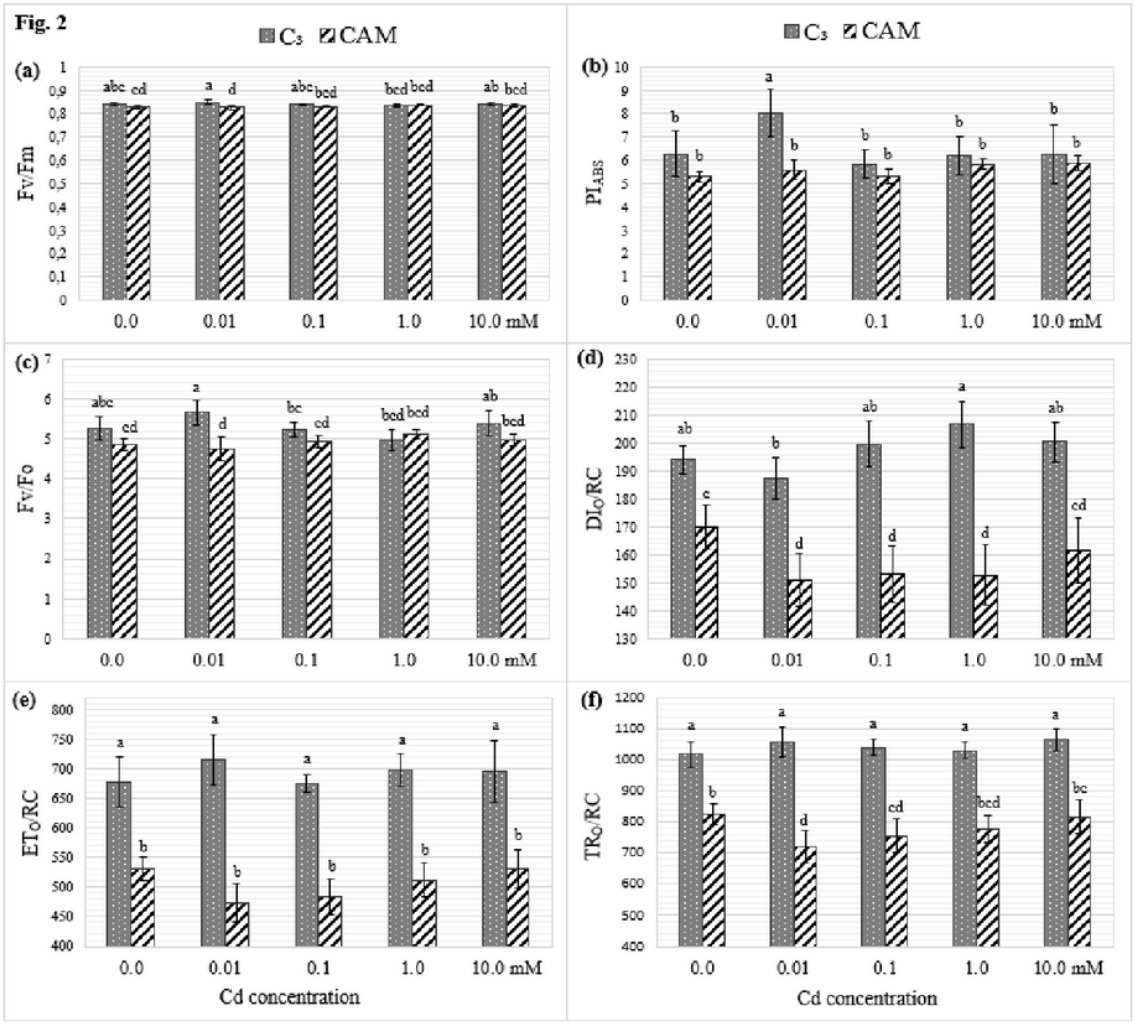
# Figures




## Figure 1

Transpiration rate in the light (a), in the dark (b), net photosynthesis - $P_N$ (c), and respiration (d) in *Mesembryanthemum crystallinum* plants performing $C_3$ and CAM photosynthesis treated with 0 (control), 0.01, 0.1, 1.0, 10.0 mM Cd concentrations for 8 days. Bars represent mean values with standard

deviation. Different letters indicate statistically significant differences at $p \leq 0.05$ (ANOVA, Duncan's test) for n = 4.




## Figure 2

Maximum PSII quantum yield - Fv/Fm (a), performance index calculated based on energy absorption - $PI_{ABS}$ (b), maximum efficiency of water splitting complex on the PSII side - Fv/Fo (c), nonphotochemical fluorescence quenching calculated per reaction center (RC) - $DI_0$/RC (d), electron transport through carriers located in the electron transport chain after $Q_A$ per reaction center - $ET_0$/RC (e), and flow of

retained photons (reducing $Q_A$) in PSII per reaction center - $TR_0$/RC (f) in *Mesembryanthemum crystallinum* plants performing $C_3$ or CAM photosynthesis treated with 0 (control), 0.01, 0.1, 1.0, 10.0 mM Cd concentrations for 8 days. Bars represent mean values with standard deviation. Different letters indicate statistically significant differences at $p \leq 0.05$ (ANOVA, Duncan's test) for n = 12.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial.docx