Photosynthetic response of cabbage in cadmium-spiked soil

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Abstract

Efficiency of a photosynthetic apparatus in plants growing in cadmium-spiked soil as well as plant biomass and cadmium accumulation were assessed in two cabbage cultivars (early and late) in a long-term pot experiment. Cadmium concentrations were 10 and 40 mg(Cd) per kg of dry mass of soil. The early cultivar showed a dose-dependent and stable decrease in SPAD index, while in plants of the late cultivar the response was reversible and less pronounced. A huge drop in the effective quantum yield of PSII photochemistry, photochemical quenching, nonphotochemical quenching, and electron transport rate in the third week of vegetation indicated susceptibility of the early cultivar to cadmium, contrary to the late one. Fresh mass of plants of the early cultivar was diminished by both doses of Cd. For the late cultivar, there was no change in these parameters, indicating its possible usability for phytoremediation, provided the reclaimed area is fenced and protected. Particular attention should also be paid towards late cultivars cabbage farming in areas with a high Cd content. On the other hand, the early cultivar may serve as a bioindicator of Cd-contamination.

Additional key words: abiotic stress; *Brassica oleracea*; crop productivity; chlorophyll content; chlorophyll fluorescence.

Introduction

Cadmium (Cd) is one of the most toxic heavy metals. Its soil concentration depends on the parent rock but it usually exceeds acceptable limits due to anthropogenic activity (Simmons *et al.* 2005, Qu *et al.* 2013). It is supplied to soil, air, and water mainly with effluents from industry, mining, burning, and waste leakage, as well as by an application of phosphate and sewage sludge (Hsu *et al.* 2006, Moradi and Ehsanzadeh 2015). Mean concentration of cadmium in agricultural soils of Poland equals *ca.* 0.15 mg kg^{-1}(dry mass, DM), but in regions with intense mining and smelting activities it may vary from approx. 3–40 mg kg^{-1}(DM) (Kandziora-Ciupa *et al.* 2013, Bączek-Kwinta *et al.* 2019). In the roadside soils of Iran, cadmium content was reported to reach 2–3 mg kg^{-1}(DM) (Samani Majd *et al.* 2007), and in paddy soils in China, where metal ore tailings were located, it was approx. 50 mg kg^{-1}(DM) (Zhai *et al.* 2008).

Due to their high mobility and toxicity even at relatively low concentrations, divalent cadmium ions are extremely dangerous not only to soil microbiota and vegetation but,

through the food chain, also to humans (Anjum *et al.* 2016). Being highly mobile within phloem, cadmium is translocated into different plant organs, interfering with their metabolic processes (photosynthesis, respiration, nitrogen metabolism). This results in reduced growth and low biomass accumulation (Epstein and Bloom 2005, He *et al.* 2015).

Cd acts as the most potent inhibitor of photosynthetic activity (Moradi and Ehsanzadeh 2015, Sitko *et al.* 2017). Chlorophyll (Chl) loss or disturbances of its biosynthesis and carbon fixation by a leading photosynthetic enzyme, Rubisco, are particularly sensitive processes (Kučera *et al.* 2008). Cd inhibits Chl biosynthesis, enhances its degradation at heme level, hampers photochemical and carboxylation reactions of photosynthesis, and disrupts chloroplast metabolism (Wang *et al.* 2009, Sarangthem *et al.* 2011). Various studies indicated that Cd ions affect both donor and acceptor sites of PSII, leading to uncoupling of electron transport in the chloroplasts. In general, PSII functioning is inhibited to a much greater degree than that of PSI, and Cd exerts multiple effects on PSII (Kučera *et al.*

2008, Amirjani 2012). On the donor site, Cd inhibits oxygen-evolving cycle and, consequently, O_2 generation. It also diminishes the number of oxygen-evolving complexes (Bazzaz and Govindjee 1974, Vassilev *et al.* 2004, Janeczko *et al.* 2005), while on the acceptor site, it inhibits electron transfer from Q_A^- to Q_B^- (Sigfridsson *et al.* 2004). Adverse effects of Cd are also visible in carboxylation phase of photosynthesis (Krantev *et al.* 2008). Stomatal conductance and index, transpiration, and net photosynthetic rate are affected, as well as enzymes of dark phase of photosynthesis (Rivera-Becerril *et al.* 2002, Burzyński and Żurek 2007).

Metal accumulation in plant tissues is possible due to different mechanisms, such as binding metals in the cell walls, their chelation and detoxification by cellular organic compounds (organic acids, phytochelatins), deposition in metabolically inactive organelles (vacuoles), or external tissues (Kučera *et al.* 2008). These mechanisms are the basis of phytoremediation, *i.e.*, using plants to extract, sequester, and/or detoxify pollutants. Some *Brassicaceae* plants have huge potential for such techniques due to their hyperaccumulating properties (Milner and Kochian 2008, Mudgal *et al.* 2010, Tang *et al.* 2016, Sitko *et al.* 2017). Some are not hyperaccumulators but can be useful too (Radulescu *et al.* 2013, Goswami and Das 2015, Bernard *et al.* 2018). Cabbage (*Brassica oleracea* subsp. *capitata* f. *alba*), a well-known representative of *Brassicaceae*, grows rapidly and produces large biomass in a small volume. It is also perfectly adapted to the climate of many countries. Studies on bioaccumulation of metals by cabbage revealed accumulation of Cd in this plant growing in various locations (Sękara *et al.* 2005). Reports by Ciura *et al.* (2005), Bączek-Kwinta *et al.* (2011a) and Kusznierewicz *et al.* (2012) confirmed usefulness of white cabbage for phytoextraction in the soil contaminated with Cd.

The parameters of fluorescence of Chl *a* (CF) provide *in vivo* assessment of the photosynthetic apparatus activity under stress. The measurements are noninvasive, and often used as an alternative for gas-exchange measurement of photosynthesis (Kouřil *et al.* 2003, Sitko *et al.* 2017). CF parameters are widely used in ecophysiological and agricultural studies (Sofo *et al.* 2009, Murchie and Lawson 2013). The aim of our work was to establish to what extent CF parameters and photometric assays of Chl pool (SPAD) reflect cabbage sensitivity to Cd stress. A secondary aim was to combine the above data with data on Cd accumulation and plant biomass to evaluate the species usefulness for phytoremediation or Cd bioassessment in the field. The range of Cd content in the soil was high and exceeded permissive values set out in framework guidelines for agriculture (Kabata-Pendias *et al.* 1995). A strong point of our study was its large scale and duration. We succeeded in emulating the actual field conditions much better than in hydroponics, and our report provides practical information that can be used in further field trials.

Materials and methods

Plant material: The experiment was performed in a greenhouse (phytotron) of the University of Agriculture in Kraków and involved two cabbage cultivars (*Brassica oleracea* ssp. *capitata* f. *alba* L.) – early cv. 'Ditmarska Najwczesniejsza' and late cv. 'Kamienna Głowa'. Seeds were treated with seed dressing T 75/DS/WS (70% Thiuram), and then sown at the same time into commercial garden soil in 100-cm³ volume pots. Seedlings of both cultivars grew at 5–15°C/15–20°C night/day temperature and were transplanted into large pots of 10-dm³ capacity at the stage of 6–8 leaves. About 10 kg of local soil taken from an experimental field of the University of Agriculture in Kraków, Poland, were placed in every pot. The soil granulometric composition involved: humus horizon, 19% sand, 5% coarse silt, 41% fine silt, 24% coarse silt clay, 6% clay fine silt, and 5% colloidal clay (pH 7.0). Further vegetation of all plants was carried out in a greenhouse, at 14/10 h (day/night) photoperiod with additional lighting on cloudy days, temperature 20–25°C/17–20°C (day/night), and relative humidity 30–50%. The plants were fertilized differentially according to the agricultural protocols for fast-growing early cultivars and late cultivars that grow for a long period of time: N (NO_3–N + NH_4–N) – 105 (early cultivar) and 120 (late cultivar) mg dm⁻³(DM of soil), P [Ca(HPO$_4$)$_2$] – 50 and 60 mg dm⁻³, K (KCl, 60% potassium salt) – 160 and 180 mg dm⁻³), respectively. The plants were irrigated daily with tap water to avoid wilting. Plant rotation towards different corners of the greenhouse was performed every 2–3 d to eliminate the effect of local changes in temperature, illumination or humidity. The plants grew for 91 (early cultivar) and 151 d (late cultivar).

Cd treatment: Cd concentrations were established based on previous experiments by Bączek-Kwinta *et al.* (2011a) and Kusznierewicz *et al.* (2012). The amounts exceeded the concentration of 4 mg kg⁻¹ permissible in Poland (Regulation by the Minister of Environment 2002). Cd was added to the soil 10 d before planting as $CdSO_4 \cdot 8H_2O$ (*Sigma-Aldrich*) dissolved in 100 mL of deionised water, at 10 and 40 mg(Cd) kg⁻¹(DM of soil), and mixed thoroughly with the soil. The objects will be termed Cd10 and Cd40 further in the whole manuscript. Control pots contained the same soil treated with the same volume of deionised water but without Cd. Control amount of Cd was approx. 0.4 mg kg⁻¹ (Kusznierewicz *et al.* 2012). The experiment was performed in five replications for each treatment and cultivar.

Soil and plant analysis: For metal content determination, air dried and lyophilized soil samples were mineralized in a microwave-assisted Anton Paar digestion system. Mineralization was performed at 240°C and 6 MPa for 0.5-g portions of cabbage or soil mixed with 5 mL of HNO_3 + 2 mL of H_2O_2 or 7 mL of HNO_3 as a mineralizing solution, respectively. The acidic solutions after digestion were transferred into 25-mL PMP volumetric flasks and filled up with deionised water. A *GBC SensAA* atomic absorption spectrometer (*GBC Scientific Equipment*, Australia) furnished with deuterium lamp background correction, single element hollow cathode lamps (HCL), and air-acetylene flame was used for Cd determination. In the events of particularly low Cd content, a *GBC*

SavantAA Z atomic absorption spectrometer (*GBC Scientific Equipment*, Australia) with graphite furnace atomization was employed. The sample volume injected was 10 µL and the matrix modifier (1% solution of $NH_4H_2PO_4$) volume was 5 µL. Cd HCL was used as radiation source at 228.8 nm. A calibration curve prepared with the use of AAS/ICP grade standard stock solutions (1,000 µg mL⁻¹ of Cd) was applied for quantitative analysis.

Bioaccumulation factor was calculated as follows: $BCF = C_p/C_s$, where C_p is metal concentration in plant sample [mg kg⁻¹] while C_s is metal concentration in soil sample [mg kg⁻¹]. Plants can be accumulators if BCF > 1, when BCF = 1 there is no uptake, and if BCF < 1, then a plant can be an excluder (Baker 1981, Cluis 2004).

Relative Chl content (SPAD) and parameters of fluorescence of Chl *a* (CF): SPAD index was measured photometrically in mature, fully developed leaves with a portable Chl meter *SPAD-502 Plus* (*Konica Minolta*, Japan). Leaves at similar stage of development (third to fifth leaf from the top) were considered. Chl *a* and *b* absorb red but not infrared light. The meter determines absorbance in these regions, and provides numerical values of SPAD readings (maximum is 60), which are proportional to Chl content (Konica Minolta 2009). The results are expressed as 'Chl index' or 'greenness index'. The parameters of fluorescence of Chl *a* were measured with *FMS-2* fluorometer (*Hansatech*, United Kingdom) in the same leaves as SPAD. After 20 min of dark adaptation, a saturating light pulse [10,000 µmol(photon) m⁻² s⁻¹ for 0.9 s] was used to obtain F_m. Next, the leaf was irradiated with actinic light [1,500 µmol(photon) m⁻² s⁻¹] for 270 s.

Then, the saturating light pulse was used again. The source of a modulation beam (duration pulses 1.8 µs, 2.3 kHz) was an amber LED [peak wavelength of 594 nm, PPFD *ca*. 0.05 µmol(photon) m⁻² s⁻¹]. Actinic and pulse irradiations were provided by a halogen lamp (20 W). The signal detector was a PIN photodiode with a long-pass filter (> 700 nm), and the sampling rate was 10–20 kHz (depending on the instrument mode). Parameters of the maximum photochemical efficiency of PSII (F_v/F_m), the quantum efficiency of photochemical reactions in PSII (Φ_{PSII}), the photochemical quenching of PSII (q_P), the nonphotochemical quenching (NPQ), and the efficiency of electron flow rate (ETR) were automatically calculated according to Genty *et al.* (1989) and Schreiber *et al.* (1986). Stern-Volmer nonphotochemical quenching was expressed as $NPQ = (F_m – F_m')/F_m'$ (Bilger and Björkman 1991).

Statistical analysis: To investigate normality of distribution, *Kolmogorov-Smirnov*'s test was used. Then analysis of variance (*ANOVA*) with repeated measurements and *Duncan*'s test ($n = 5$, $P=0.05$) were performed. The differences were considered significant if P was at least ≤ 0.05. All the analyses were carried out using *Microsoft Office Excel 2007* and *Statistica 12.0* package (*SPSS*, USA).

Results

SPAD and CF parameters: SPAD index depended primarily on the growth stage ('date' factor) and cultivar (Fig. 1*A,B*; Table 1). It increased with time and plants of the late cultivar showed higher values of the parameter than those of the early one. Both the 'treatment' factor
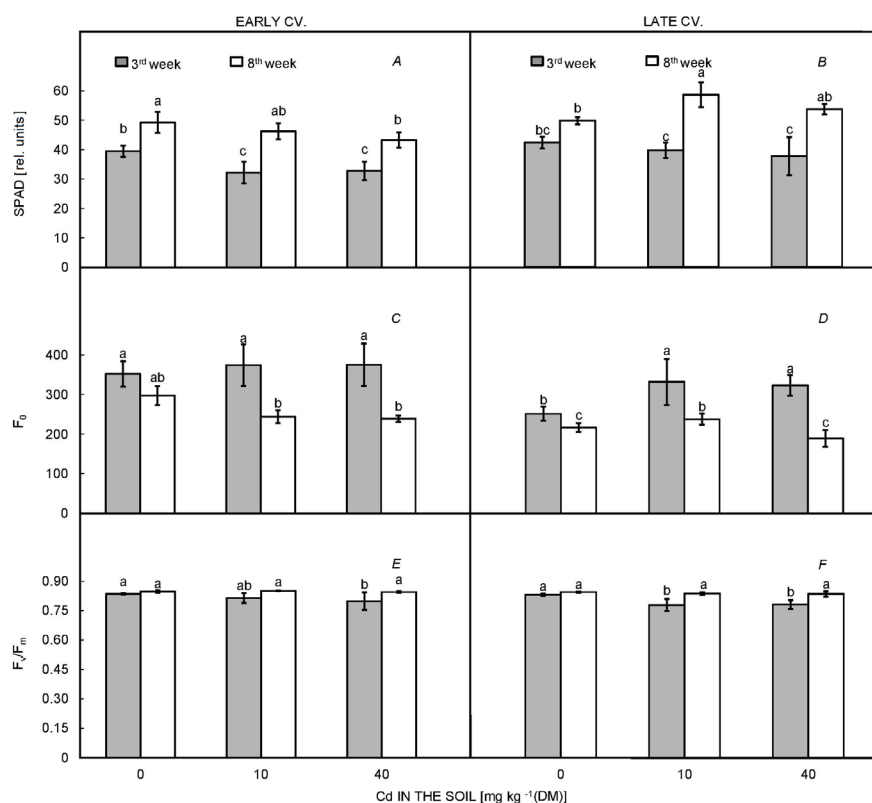
Fig. 1. The influence of cadmium on SPAD values (*A,B*) and chlorophyll fluorescence (CF) parameters (*C–F*) of leaves of white cabbage at the 3rd and 8th weeks of growth. SPAD – Soil-Plant Analyses Development (greenness index), F_0 – minimal fluorescence yield at the dark-adapted state, F_v/F_m – photochemical efficiency of PSII at the dark-adapted state. $n = 5$, means labelled with *the same letter* are not significantly different (*P*<0.05; *Duncan*'s test).

Table 1. The *F*- and *p*-values of *ANOVA* (design with repeated measurements) expressing the influence of cadmium [0, 10, and 40 mg kg⁻¹ (DM) above the natural content] on greenness index (SPAD) and chlorophyll fluorescence parameters of leaves of two cultivars of cabbage. SPAD – Soil-Plant Analyses Development (greenness index), F_0 – minimal fluorescence yield at the dark-adapted state, F_v/F_m – photochemical efficiency of PSII in the dark-adapted state, Φ_{PSII} – effective quantum yield, q_P – photochemical quenching yield, q_P – photochemical quenching coefficient, NPQ – Stern-Volmer nonphotochemical quenching of maximal fluorescence, ETR – linear electron transport rate, (e⁻) – electron. * – $\alpha < 0.05$.

Factor/Interaction	SPAD		F_0		F_v/F_m		Φ_{PSII}		q_P		NPQ		ETR [mmol(e⁻) m⁻² s⁻¹]	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Cultivar	40.8	0.000*	15.34	0.001*	2.88	0.107	2.7	0.116	12.9	0.002*	18.1	0.000*	2.5	0.135
Treatment	4.1	0.035*	0.63	0.541	8.36	0.003*	1.4	0.263	1.9	0.176	7.93	0.003*	14	0.000*
Cultivar × Treatment	6.4	0.008*	1.85	0.184	1.44	0.264	1.4	0.275	7.9	0.003*	14.15	0.000*	3.2	0.068
Date	195.9	0.000*	39	0.000*	35.39	0.000*	115.1	0.000*	117.7	0.000*	3.02	0.099	133.8	0.000*
Date × Cultivar	1.9	0.180	0.38	0.543	0.69	0.417	0.6	0.450	9.5	0.007*	47.28	0.000*	0.8	0.378
Date × Treatment	6.3	0.008*	2.91	0.079	3.80	0.042*	2.8	0.085	3.0	0.076	6.74	0.007*	12.5	0.000*
Cultivar × Treatment × Date	1.9	0.182	0.10	0.909	0.34	0.714	2.6	0.104	5.7	0.012*	7.27	0.005*	1.8	0.200

and 'cultivar' × 'treatment' interactions were significant, which means a decrease of the values for the early cultivar, but no significant decrease for the late one (Table 1, Fig. 1*A,B*). 'Date' × 'treatment' interaction demonstrated Cd-triggered drop or a tendency in both cultivars at the third week that ceased (and/or even turned into an increase) during further cultivation (eighth week).

We saw no impact of Cd on minimal fluorescence in the dark-adapted state (F_0) (Table 1). Cultivar dependence of F_0 was manifested as higher values in plants of the early cultivar (Table 1, Fig. 1*C*). Time-scale analysis revealed a similar course of F_0 decrease in all plants (Fig. 1*C,D*; Table 2).

The values of maximal quantum yield of PSII photochemistry (F_v/F_m) depended on the 'treatment', 'date', and their interaction (Table 1). Cd40 (early cultivar) and Cd10 and Cd40 (late cultivar) caused a decrease in F_v/F_m in the third week. Then, the values increased to the control level which remained unchanged over time (Table 1, Fig. 1*E,F*).

The effective quantum yield of PSII photochemistry (Φ_{PSII}) depended on the 'date' only, which means the values increased in time irrespectively of the cultivar or treatment (Table 1, Fig. 2*A,B*). However, *Duncan*'s test revealed the difference between Cd-treated plants and the control of the early cultivar (Fig. 2*A*).

In the case of photochemical quenching (q_P), *ANOVA* indicated significance of all factors but 'treatment' and 'date' × 'treatment' (Table 1, Fig. 2*C,D*). Hence, mean values of q_P were lower for the early than for the late cultivar ('cultivar' factor), due to a sharp decrease experienced in the third week by the early plants *vs.* control ('cultivar' × 'treatment'). Similarly to previously described parameters, we saw an increase in q_P for the eighth week as compared to the third week ('date' significant, Table 1, and the *Duncan*'s test, Fig. 2*C,D*).

For NPQ, *ANOVA* indicated the impact of all factors except for 'date' (Table 2). As in the case of q_P, plants of the early cultivar demonstrated lower NPQ than those of the late one (Fig. 2*E,F*; respectively). This was caused mostly by a large drop in the presence of Cd (Fig. 2*E*), as the control values of NPQ were similar for both cultivars (Fig. 2*E,F*).

Electron flow rate (ETR) depended on the 'treatment', 'date', and their interaction (Table 1). In the plants of the early cultivar (Cd40), ETR diminished by one third of control in the third week but increased during the eighth week and doubled that of the control (Fig. 2*G*). For plants of the late cultivar, the ETR pattern during the third week resembled that of Φ_{PSII} and q_P, and during the eighth week the values increased (Fig. 2*G,H*).

Plant biomass and Cd accumulation: Head size of the plants in the early cultivar diminished in the presence of both doses of Cd to similar extent (80–90% of control), while in the late cultivar similar fresh mass was obtained irrespectively of the treatment (Table 2).

Bioavailable Cd and bioaccumulation factor (BCF) were similar in both controls (Table 2). For Cd10 treatment, bioavailable Cd equaled 1,357 and 1,168% of the control for the early and late cultivar, respectively. Interestingly,

Table 2. Biomass of heads and cadmium accumulation by cabbage grown in unspiked soil and the soil spiked with cadmium. The natural content of cadmium was approx. 0.4 mg kg^{-1}(DM). The early cultivar was grown for 91 d, the late one for 151 d. The data of different treatments within the cultivar marked by *the same letter* are not significantly different at *P*<0.05; *Duncan'*s test. Cd treatment – content of Cd in the soil above the natural value, FM – fresh mass, DM – dry mass.

| Cd treatment [mg kg^{-1}(DM)]	Biomass of heads [kg FM] and in relation to control [%]		Bioavailable Cd [mg kg^{-1}(head DM)] and in relation to control [%]		Bioaccumulation factor [mg Cd(heads) mg Cd(soil)$^{-1}$] and in relation to control [%]	
	Early cultivar	Late cultivar	Early cultivar	Late cultivar	Early cultivar	Late cultivar
0	0.737 ± 0.081[a] 100%	1.215 ± 0.095[a] 100%	0.37 ± 0.01[a] 100%	0.38 ± 0.16[a] 100%	1.11 ± 0.26[a] 100%	1.19 ± 0.33[a] 100%
10	0.609 ± 0.070[b] 82%	1.441 ± 0.152[a] 119%	5.02 ± 0.35[b] 1,357%	4.44 ± 0.83[b] 1,168%	0.49 ± 0.04[b] 44%	0.44 ± 0.04[b] 37%
40	0.654 ± 0.070[b] 88%	1.219 ± 0.231[a] 100%	8.56 ± 0.90[c] 2,314%	18.7 ± 1.70[c] 4,921%	0.21 ± 0.02[c] 19%	0.46 ± 0.06[b] 39%
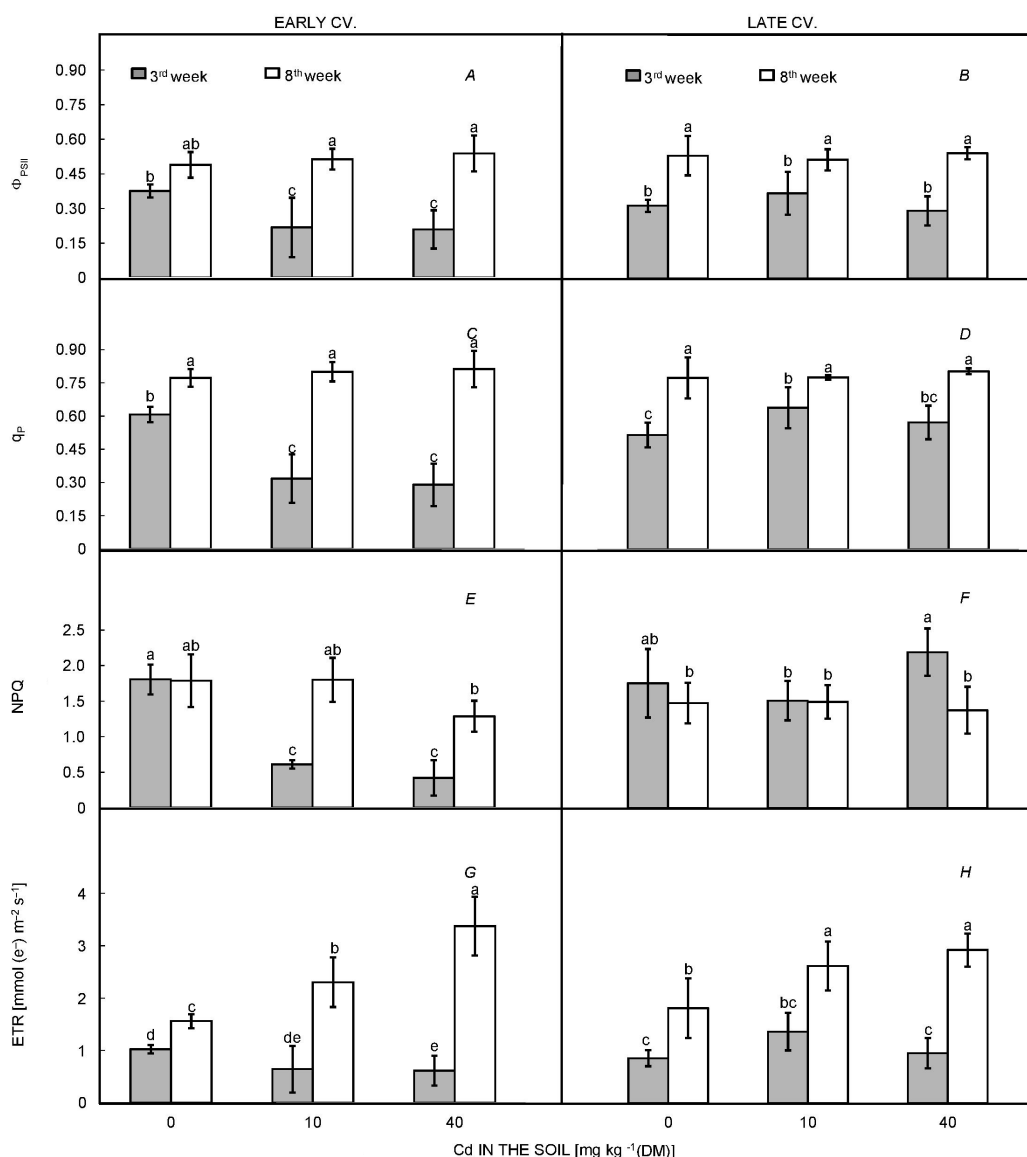
Fig. 2. The influence of cadmium on chlorophyll fluorescence parameters of leaves of white cabbage at the 3rd and 8th weeks of growth. Φ_{PSII} – effective quantum yield, q_P – photochemical quenching coefficient, NPQ – Stern-Volmer nonphotochemical quenching of maximal fluorescence, ETR – linear electron transport rate. *n* = 5, means labelled with *the same letter* are not significantly different (*P*<0.05; *Duncan*'s test).

the BCF values diminished in plants exposed to Cd10 (44 and 37% of respective control). For Cd40, a great increase in bioavailable Cd occurred (2,314 and 4,921% of respective controls), but BCF was lower than for Cd10 and the values were 19 and 39% for early and late cultivar, respectively (Table 2).

Discussion

SPAD and CF parameters: The greenness index measured with different devices is a popular parameter used in studies on plant response to different stress factors (Haripriya Anand and Byju 2008, Sękara *et al.* 2016, Bączek-Kwinta *et al.* 2019). The method is noninvasive, which was important in the described experiment especially at the beginning of vegetation (in the third week). Moreover, the measurements can be taken in the field, and we were interested whether the values of SPAD matched those of CF depending on Cd concentration. Our results revealed a stable downward trend in SPAD index in the early cultivar for both Cd doses, indicating diminished pool of Chl. Even though we did not perform chemical determination of Chl, its loss and mechanisms of this loss are well known in the case of cadmium stress. These mechanisms include Chl degradation or disturbances of its synthesis or inhibition of cell division and replication of chloroplasts (Baryla *et al.* 2001, Nikolić *et al.* 2008). Cd inhibits reduction of protochlorophyllide and synthesis of aminolevulinic acid (ALA), a specific precursor of Chl biosynthesis (Bhattacharjee and Mukherjee 2003, Sarangthem *et al.* 2011). The decrease in Chl content can also be due to increased iron uptake in the presence of Cd. Iron substitutes magnesium and heme is produced instead of Chl (Zhou and Qiu 2005).

Greenness index often inversely corresponds with F_0, namely, higher greenness index and chemically assayed Chl content correlate with lower F_0 values (Baker 2008, Borek *et al.* 2016). The downward trend in SPAD readings for the late cultivar ceased over time, and the time course of changes in F_0 was opposite to that of SPAD. We can therefore assume that Chl biosynthesis was restored in these plants contrary to those of the early cultivar.

A slight decline in F_v/F_m in the third week with increasing concentration of Cd indicated stress exposure of the photosynthetic apparatus (Muniz *et al.* 2014, Moradi and Ehsanzadeh 2015). However, the ratio increased over time, so we can expect the response is reversible and plants are capable of acclimating to such conditions. Temporary declines of F_v/F_m and SPAD suggest that Cd not harmfully affected the symplast which implies vacuolar sequestration mechanisms and protection against Cd-triggered oxidative stress (Carrier *et al.* 2003, Nikolić *et al.* 2008, Mohamed *et al.* 2012). However, even distinct changes in F_v/F_m may be insufficient to accurately assess functioning of the photosynthetic apparatus. Therefore, it is necessary to take other values into account (Bączek-Kwinta *et al.* 2011b, Borek *et al.* 2016).

Low Φ_{PSII} values in the early cultivar plants grown in Cd-contaminated soil confirmed disturbances of photosynthetic processes and diminished ratio of quanta used in photochemical processes to the total number of absorbed PAR quanta (Genty *et al.* 1989). This may correlate with diminished rate of CO_2 uptake in the leaves and result directly in lower carbon assimilation (Kalaji *et al.* 2014).

A decrease in q_P values in the plants of the early cultivar confirmed their sensitivity to Cd and indicated a closure of the PSII reaction centers during photosynthesis saturated by actinic radiation (Maxwell and Johnson 2000).

The nonphotochemical quenching of PSII (NPQ) comprises the processes that convert part of the energy absorbed in the light phase of photosynthesis into heat (Maxwell and Johnson 2000). Intensification of these processes occurs in the presence of very intense light or other stress factors. The increase in NPQ reflects energy dissipation by heat *via* the carotenoids (Car) of the xanthophyll cycle (VAZ) and other Cars, as well as by conformational changes of PSII antennae (LHC) (Havaux and Kloppstech 2001, Szabó *et al.* 2005, Sofo *et al.* 2009). In this study, we saw a decrease in NPQ in the third week in the plants of the early cultivar. This was surprising, because if photochemical quenching declined, the absorbed energy should have been dissipated, mainly by the Cars (Pogson and Rissler 2000). The reason might be a reduced synthesis of these pigments, which is typical of Cd stress (Jiang *et al.* 2013, Cheng *et al.* 2016, Paunov *et al.* 2018).

Similarly to the previously discussed parameters, ETR declined in the third week in the early cultivar. This suggests a disruption in functionality of the thylakoid-bound electron transport chain from PSII to PSI (Papadakis *et al.* 2007). Considering the above-described declines, it can be inferred that the photosynthetic apparatus of plants of the early cultivar was under considerable stress at the beginning of the growing season. The plants of the late cultivar showed a weaker response. Such differences result probably from faster growth of the early cultivar plants *vs.* those representing the late one (data not shown). ETR was the only parameter, the values of which in the eighth week increased along with growing Cd concentrations in soil (compared to control plants). This suggests intensification of electron flow between PSII and PSI. It is also possible that other processes involving electrons, such as photorespiration, Mehler reaction, and reduction of nitrates occurred (Fracheboud and Leipner 2003).

Overall, the CF results confirmed that Cd affected the photosynthetic apparatus in a different way in both cultivars, which was reflected in different pattern of changes in studied parameters. The parameters which revealed distinct changes in response to Cd treatment were Φ_{PSII}, q_P, NPQ, and ETR, therefore they can be used as markers of the physiological changes induced by Cd treatment.

Plant biomass and Cd accumulation: Previous reports (Bączek-Kwinta *et al.* 2011a, Kusznierewicz *et al.* 2012) showed that cabbage plants, especially those of the late cultivar, are capable of growing in Cd-spiked soil, tolerating Cd, and accumulating this element in their heads. This study corroborated these claims. Despite large amount of soil used in the experiment, FM of all heads was rather low, which is typical of greenhouse conditions (data not shown). In the field, the yield of 150 to 300 tons per hectare is

achievable, with estimated output of Cd up to 180 to 360 g per ha, according to Kusznierewicz *et al.* (2012). When we compare phytoremediative properties of cabbage to other edible plants, the potential uptake of Cd by cabbage seems higher than of, *e.g.*, wheat or Cd-hyperaccumulator chard (*Beta vulgaris* L. var. *cicla*) (Shumaker and Begonia 2005, Song *et al.* 2012). Yet, it depends on the cultivar. The early one accumulated less Cd than the late one, and its biomass was lower. Moreover, smaller bioaccumulation factor indicates that the plants did not allow Cd to distribute in heads, so either Cd uptake from the soil was limited, or its accumulation in roots occurred to a bigger extent than in the plants of the late cultivar (Baker 1981, Cluis 2004). Kusznierewicz *et al.* (2012) claimed that since plants of the early cultivar may be planted twice per agricultural season, the phytoremediation results may be similar to those of the late cultivar, even though such a solution would be more cost- and labor-consuming. Metal uptake may depend on soil composition and pH (McBride 1989, Melo *et al.* 2014, Bernard *et al.* 2018); hence, implementation of proper agricultural treatments (such as intense fertilization of the late cultivars) is necessary. An environmental issue should be also considered, namely potential transfer of Cd from the spiked plants into small herbivores leading to further release of Cd to the trophic chain (Hladun *et al.* 2015). To prevent the entrance of larger animals and humans, the area subjected to phytoremediation should be fenced and clearly labelled. Particular attention should also be paid towards agricultural crops of late cabbage in areas with high Cd content.

Conclusion: In the presence of Cd, the photosynthetic apparatus in the early cultivar of cabbage operated less efficiently than in the late one. The changes occurred rapidly, and they were reflected in the yield as well as diminished biomass of heads. This, together with lower bioaccumulation factor of Cd in the early cultivar plants, allows us to identify the early cultivar as useful for the bioassessment of soil contamination. The late cultivar can be recommended for phytoremediation of Cd-contaminated soils, due to less intense response of the plants reflected by high head biomass and high bioaccumulation factor. The parameters of fluorescence of Chl *a* considerably affected by Cd treatment involved Φ_{PSII}, q_P, NPQ, and ETR, hence they may be the markers of Cd-triggered physiological changes in cabbage plants.

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