

Quantify the Response of Purslane Plant Growth, Photosynthesis Pigments and Photosystem II Photochemistry to Cadmium Concentration Gradients in the Soil¹

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Abstract—The cadmium (Cd), being a widespread soils pollutant and one of the most toxic heavy metals in the environment, adversely affects sustainable crop production and food safety. Pot experiment was conducted to quantify and simulate the response of purslane (*Portulaca oleracea* L.) plants to Cd toxicity. The purslane germinated seeds were cultivated in twelve Cd concentrations (from 0 to 300 mg/kg of Cd in soil) for six weeks and then some growth characteristics, photosynthesis pigments, and chlorophyll *a* fluorescence parameters were measured. The influence of Cd gradients in the soil on all growth parameters, photosynthesis pigments and chlorophyll *a* fluorescence parameters (except F_m and carotenoid content) were described by a segmented model. Furthermore, F_m and carotenoid contents were fitted to a linear model. The growth characteristics, chlorophyll content, photosynthetic pigments and some parameters of chlorophyll *a* fluorescence such as F_v , F_v/F_m , Y(II) and ETR decreased when Cd concentration increased. In contrast, F_0 , Y(NPQ) and Y(NO) increased and F_m was not significantly affected. In general, most variations in the studied parameters were recorded with low concentrations of cadmium, which ranged from 0 to 125 mg/kg. Also, the growth characteristics (especially stem, leaf, and shoot dry weights) were more sensitive to Cd contamination than other parameters. Moreover, among chlorophyll fluorescence parameters, Y(NPQ) was the most sensitive to Cd concentration gradients in the soil that can be due to disturbances of antennae complex of PSII.

Keywords: *Portulaca oleracea*, Cd toxicity, chlorophyll content, chlorophyll fluorescence, segmented model

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INTRODUCTION

In recent years, agroecosystems particularly in developing countries have been contaminated with heavy metals due to various human and natural activities [1]. Some of these metals such as zinc, copper, manganese, nickel, and cobalt are micronutrients and are essential for healthy plant growth, while others such as cadmium, lead, and mercury have unknown biological function and are toxic to plants [1]. However, plants take them up rapidly when present in the growing medium [2]. The cadmium is a widespread

pollutant of soils and one of the most toxic heavy metals in the environment. It does not only adversely affect sustainable crop production and food safety [3], but also threatens agricultural land quality [4]. It is well known that Cd is relatively mobile in soil and plants and can be highly toxic even at low concentrations [4].

In plants, Cd causes damage to the photosynthetic apparatus, decrease Rubisco activity in the Calvin cycle, reduce carbohydrate assimilation [5, 6], damage photosystems I and II [5–8], and thus reduces the maximum photochemical efficiency of PSII or F_v/F_m [6] and increases non-photochemical quenching [5]. In addition, Cd decreases the levels of total chlorophyll (Chl) and carotenoid [6, 8–10], inhibits the activities of antioxidative enzymes of plants [11] and induces oxidative stress in cells [11]. Moreover, the negative effects of Cd on water and nutrient uptake have also been reported [6].

The light energy absorbed by leaf is used in the photochemistry process to drive photosynthesis, dissipated as heat or chlorophyll fluorescence from PSII.

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Abbreviations: ETR—electron transport rate; F_0 —minimum fluorescence of dark sample; F_m —maximum fluorescence of dark sample; F_v —variable fluorescence; F_v/F_m —maximum photochemical quantum yield of PSII; F_0' —minimum fluorescence of illuminated sample; F_m' —maximum fluorescence of illuminated sample; F_t —steady state fluorescence; Y(II)—effective photochemical quantum yield of PSII; Y(NPQ)—quantum yield of regulated energy dissipation; Y(NO)—quantum yield of non-regulated energy dissipation.

These three processes are in competition for excitation energy [12]. Hence, through measuring the yield of Chl fluorescence, information about changes in the efficiency of photochemistry and heat dissipation can be obtained [13]. The responses of fluorescence parameters to Cd concentration were investigated in *Oryza sativa* [14], *Solanum lycopersicum* [15], *Lactuca sativa* [6], and *Elsholtzia argyi* [7].

The purslane (*Portulaca oleracea*), as an ornamental plant, is an annual C₄ succulent plant of the Portulacaceae family. It is one of the eight most common plants in the world [16], having many medicinal values, and is an important vegetable crop in southern Europe and Asia [17]. The shoot of purslane is a rich source of ω-3 fatty acids, α-tocopherols, ascorbic acid, β-carotene, and glutathione, and large amounts of potassium and magnesium [18]. In previous study [19] the purslane plant was introduced as a heavy metal tolerant and accumulator plant; therefore, it is recommended as a candidate for use in phytoremediation programs. However, prior to this study, there was no report on the extent of cadmium tolerance, as well as quantifying the response of growth and fluorescence parameters in purslane plants to Cd concentration gradients in the soil.

MATERIALS AND METHODS

Plant materials. The seeds of *P. oleracea* L. were surface sterilized in 1% NaClO for 15 min, then rinsed six times with distilled water and germinated on wet filter paper in Petri dishes for 24 h at 28 ± 1°C. The fifteen germinated purslane seeds were cultivated in each pot and placed in a greenhouse during spring and summer under natural illumination (June-August, Sari, Iran: 36°39' N and 53°4' E) with 28/20 ± 2°C (day/night) temperature and with relative humidity of 55–65%. The pots were watered daily to maintain about 70–80% of the field water holding capacity during the whole test period. The plants were harvested six weeks after planting and height, stem diameter, leaf number and area (Digimizer software, v. 4.1.1.0, MedCalc Software, Belgium) were measured. The dry weight of samples was determined after oven drying at 72°C for 24 h.

Soil preparation. The soil used in this experiment was collected from the research field of Sari Agricultural Sciences and Natural Resources University, Sari, Iran, and mixed with sand (2 : 1, v/v). The soil samples were air-dried, sieved to pass 2 mm and then some physical and chemical properties were measured. The main properties of the soil are as follows: 29.1% sand; 38.6% silt; 32.3% clay (clay loam); 0.21% N; 9.8 mg/kg P; 251 mg/kg K; pH 7.4; EC—1.06 dS/m; 35% SP; total Cd—1.08 mg/kg. CdCl₂ · 2.5H₂O was added to soil to reach Cd concentration gradients ranging from 0 to 300 mg/kg. Then soils were placed into plastic pots and incubated at 24°C for 4 weeks,

allowing the metal to distribute into various fractions. Each treatment was replicated three times.

Measurements of photosynthetic pigments and chlorophyll contents. Photosynthetic pigments levels including Chl *a* and *b* as well as carotenoid were measured using the method described by Lichtenthaler and Buschmann [20]. Leaves fresh tissue (1.0 cm²) was extracted by incubation in methanol at room temperature for 24 h in the dark, and measured at 665.2 (*A*_{665.2}), 652.4 (*A*_{652.4}), and 470 (*A*₄₇₀) nm, using a spectrophotometer (Spekkol 1300; Analytik Jena, Germany). The contents of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoid were calculated using the equations 1 to 3, respectively:

$$\text{Chl } a \text{ (}\mu\text{g/mL)} = 16.72 A_{665.2} - 9.16 A_{652.4}, \quad (1)$$

$$\text{Chl } b \text{ (}\mu\text{g/mL)} = 34.09 A_{652.4} - 15.28 A_{665.2}, \quad (2)$$

$$\begin{aligned} \text{Carotenoid (}\mu\text{g/mL)} = & (1000A_{470} \\ & - 1.63\text{Chl } a - 104.96\text{Chl } b)/221. \end{aligned} \quad (3)$$

The chlorophyll content (SPAD value) was determined in the youngest fully expanded leaf, using a portable chlorophyll meter (SPAD-502, Minolta, Japan).

Measurements of chlorophyll *a* fluorescence. The Chl fluorescence was measured with a portable fluorometer (PAM-2500, Walz, Germany) according to Genty et al. [21]. The leaf samples were dark-adapted for 30 min using leaf-clip holder (2030-B, Walz). The minimum fluorescence intensity (*F*₀) and maximum fluorescence intensity (*F*_m) were measured in dark-adapted leaves. The variable fluorescence (*F*_v) and maximum photochemical quantum yield of PSII (*F*_v/*F*_m) were calculated as shown in equations (4) and (5), respectively [22]. Moreover, the light-adapted measurements in actinic light provided minimum (*F*'₀), maximum (*F*'_m), and steady state fluorescence (*F*'_t). The fluorescence parameters were determined in both light- and dark-adapted leaves. Effective photochemical quantum yield of PSII [Y(II)], quantum yield of regulated energy dissipation [Y(NPQ)] and quantum yield of non-regulated energy dissipation [Y(NO)] were calculated using Equations 6 to 8, respectively [23]. Electron transport rate (ETR) was estimated using the method of Genty et al. [21] (equation 9).

$$F_v = F_m - F_0, \quad (4)$$

$$F_v/F_m = (F_m - F_0)/F_m, \quad (5)$$

$$Y(\text{II}) = (F'_m - F'_t)/F'_m, \quad (6)$$

$$Y(\text{NPQ}) = (F'_t/F'_m) - (F_t/F_m), \quad (7)$$

$$Y(\text{NO}) = F'_t/F'_m, \quad (8)$$

$$\text{ETR} = Y(\text{II}) \times \text{PFDa} \times 0.5. \quad (9)$$

Statistical analysis. The experiment was arranged in completely randomized design with three repli-

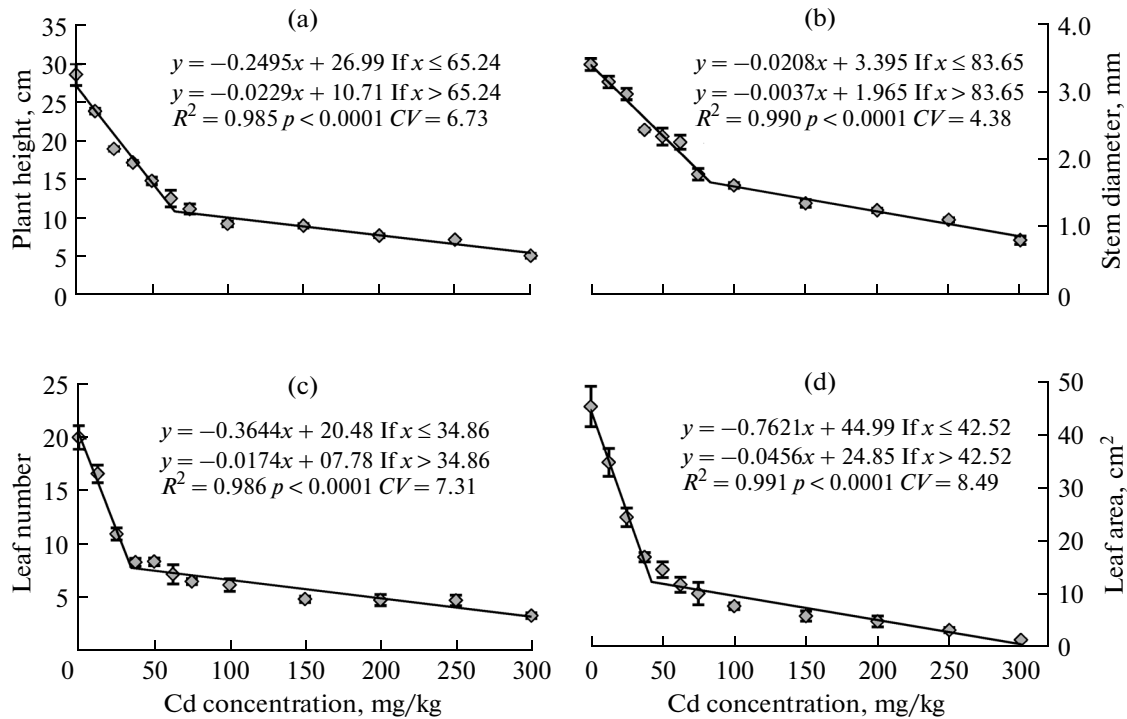


Fig. 1. The response of plant height (a), stem diameter (b), leaf number (c), and leaf area (d) to Cd concentration gradients in the soil using the segmented model (eq. 10). Values are mean of three replicates and bars indicate \pm SE ($n = 3$).

cat. The statistical analysis was performed using the SAS v. 9.1 [24] and graphs were drawn using Excel software. A segmented model (equation 10) was used to quantify the response of purslane plants growth, physiological (except carotenoid) attributes and chlorophyll fluorescence (except F_m) parameters to Cd concentration gradients [25]:

$$y = b_1x + a, \quad \text{if } x \leq x_0,$$

$$y = (b_1x_0 + a) + b_2(x - x_0), \quad \text{if } x > x_0, \quad (10)$$

where y —the predicted value for given characteristics, a —the constant value in zero concentration of Cd, x —the cadmium concentration in the soil, x_0 —the turning point between two phases, b_1 and b_2 —the slop of plant parameter variation (decrease or increase) in phases 1 and 2, respectively. The carotenoid and F_m were fitted to a linear regression model (equation 11):

$$y = b_1x + a. \quad (11)$$

RESULTS AND DISCUSSION

Growth Parameters

As shown in Figs. 1a and 1d, a segmented model was fitted to describe the purslane growth characteristics at different Cd concentrations. Plant height, stem diameter, leaf number, and leaf area decreased linearly, when cadmium concentration increased. These growth parameters decreased by 80, 75, 85, and 98%, respectively, in 300 mg/kg of Cd in the soil as compared to the control (0 mg/kg of Cd).

In order to detect the relationship between stem, leaf, and shoot dry weights with Cd concentration gradients, the segmented model was fitted to each data (Fig. 2 and table). The stem, leaf and shoot dry weights decreased rapidly and linearly when cadmium concentration increased to 40.22 mg/kg of soil, and then slightly decreased up to 300 mg/kg of Cd in the soil.

The toxic and inhibitory effects of Cd on plant growth have been widely studied in different plant spe-

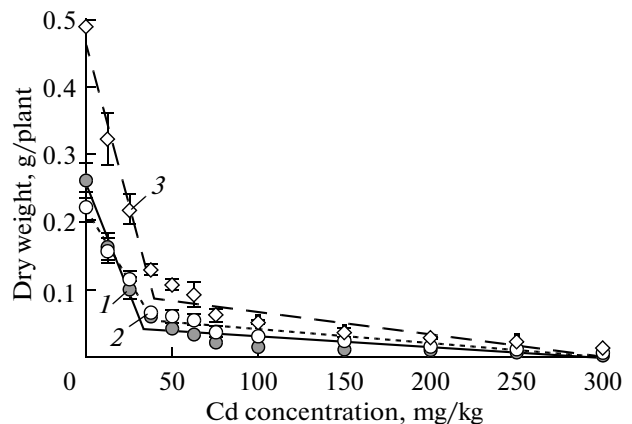


Fig. 2. The response of stem (1), leaf (2), and shoot (3) dry weight to Cd concentration gradients in the soil using the segmented model (eq. 10). Values are mean of three replicates and bars indicate \pm SE ($n = 3$). See table for more details.

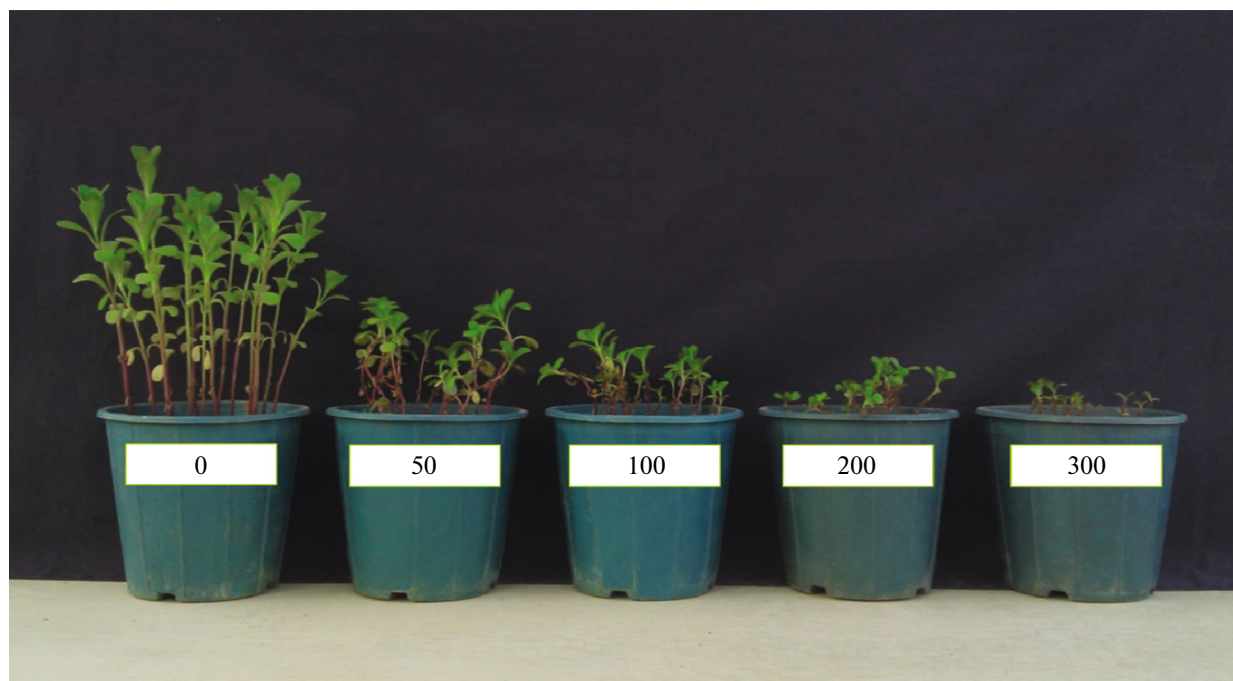


Fig. 3. The response of purslane plants growth to Cd concentrations (mg/kg) in the soil 6 weeks after sowing.

cies [5–9, 26]. Also, these results are consistent with the results of previous studies, for example Naz et al. [26] in purslane (*P. oleracea*), Shahabivand et al. [9] in wheat (*Triticum aestivum*) and Xue et al. [8] in soybean (*Glycine max* L.) who reported a reduction in root and shoot weights, plant height and stem diameter, when Cd concentrations increased in the soil. In the present study, the highest inhibitory effect of Cd concentration gradients was recorded for shoot dry weight (Figs. 1 and 2). Similarly, Chen et al. [27] observed that Cd contamination up to 24 mg/kg in the soil caused a significant decline in the shoot dry weight of pakchoi (*Brassica campestris* ssp.) and mustard (*Brassica juncea* Czernajew) plants. Also, the reduction of shoot dry weight in lettuce [6] and soybean [8] was 73 and 29%, respectively, when the plants were exposed to 50 μ M and 100 μ M (approximately equivalent to 5.62 mg/L and 11.24 mg/L) of Cd in the grow-

ing culture solution. However, in the present study, the reduction of shoot dry weight was significant when Cd concentration increased to 50–300 mg/kg in the soil (Figs. 2 and 3).

Photosynthetic Pigments

Figure 4 shows the relationship between the SPAD value and photosynthetic pigments (Chl *a*, Chl *b* and carotenoid) and Cd concentrations in the soil. The influence of Cd on SPAD values ($R^2 = 0.97$), Chl *a* ($R^2 = 0.95$), and Chl *b* ($R^2 = 0.95$) were described by a segmented model. As Cd concentration increased, the SPAD value, and Chl *a*, Chl *b* contents significantly decreased (Figs. 4a–4c). The carotenoid content decreased linearly ($R^2 = 0.73$) with increasing Cd levels in the soil (Fig. 4d). Shahabivand et al. [9] similarly found a decreasing trend in the SPAD value in *T. aestivum* leaves, when Cd concentration increased in the soil. Also, using 0.9 mM of Cd in the soil (approximately equivalent to 101 mg/kg) they found that chlorophyll content markedly decreased by 96%. In the present study, however, the maximum reduction of SPAD value was 17%, recorded when purslane plants were exposed to 300 mg/kg of Cd in the soil.

The Chl *b* content was more sensitive than Chl *a* to Cd concentration gradients in the soil. When 300 mg/kg of Cd was added to the soil, the Chl *a* and Chl *b* contents decreased by 35 and 41%, respectively (Fig. 4). Oliveira et al. [28] also reported for soybean plants the reduction of Chl *a* and Chl *b* content by 91 and 89%, respectively, when the Cd concentration increased from 0 to 3.2 mg/L in nutrient solution.

Fitted models for stem, leaf, and shoot dry weight response to Cd concentration gradients in the soil

Stem dry weight	$y = -0.0064x + 0.258$, if $x \leq 33.29$ $y = -0.0002x + 0.049$, if $x > 33.29$ $R^2 = 0.984$, $p < 0.0001$, $CV = 16.12$
Leaf dry weight	$y = -0.0041x + 0.220$, if $x \leq 40.33$ $y = -0.0002x + 0.053$, if $x > 40.33$ $R^2 = 0.988$, $p < 0.0001$, $CV = 10.81$
Shoot dry weight	$y = -0.0094x + 0.469$, if $x \leq 40.22$ $y = -0.0003x + 0.089$, if $x > 40.22$ $R^2 = 0.987$, $p < 0.0001$, $CV = 12.81$

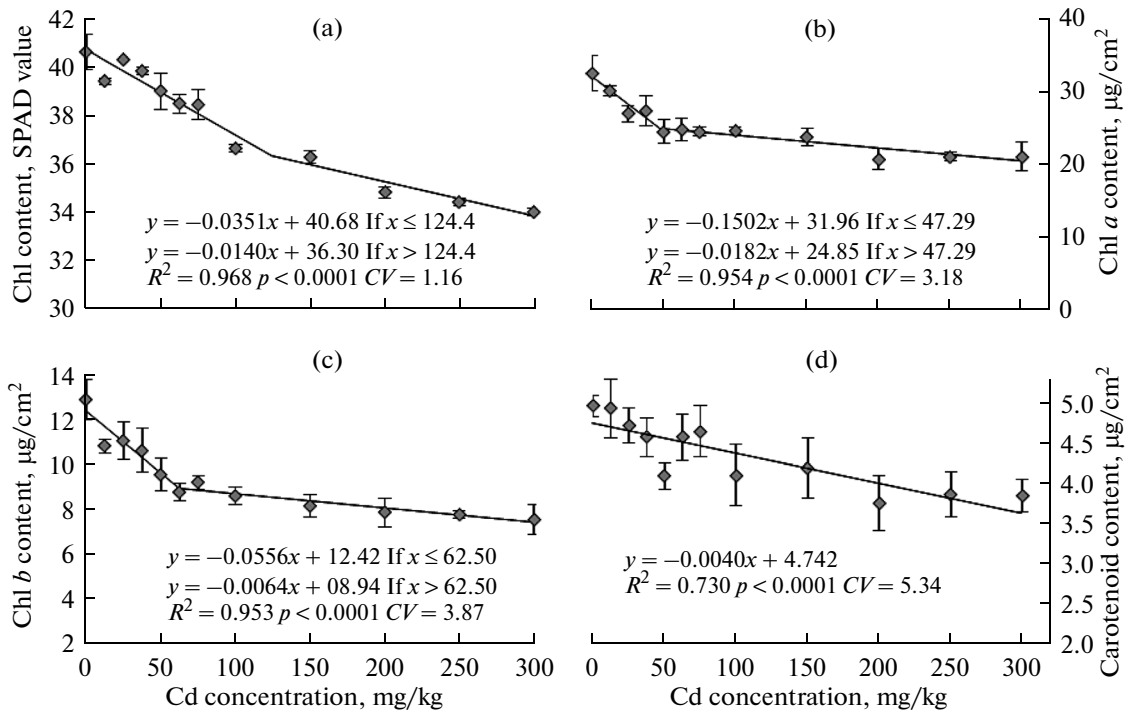


Fig. 4. The response of SPAD value (a), chlorophyll *a* (b), chlorophyll *b* (c) and carotenoid (d) contents) to Cd concentration in soil, using the segmented (eq. 10) and liner (eq. 11) models. Values are mean of three replicates and bars indicate \pm SE ($n = 3$).

Similarly, Celeste Dias et al. [6] found that the exposure of lettuce plants to 10 and 50 μM (approximately equivalent to 1.12 and 5.62 mg/L) of Cd in culture solution, significantly decreased the content of Chl *a* (32.5 and 72.5%, respectively) and Chl *b* (21 and 37.5%, respectively), as compared to the control plants. Similar results were observed in soybean [8]. When plants were exposed to 10 and 50 μM of Cd, the carotenoids content significantly increased by 43.5 and 26%, respectively, as compared to the control plants.

Chlorophyll *a* Fluorescence Parameters

As shown in Fig. 5, the response of minimum fluorescence (F_0), variable fluorescence (F_v) and maximum photochemical quantum yield of PSII (F_v/F_m) to Cd levels in the soil was described using a segmented model (Figs. 5a, 5c, and 5d), while the maximum fluorescence (F_m) data were fitted using a linear model (Fig. 5b). The parameters of F_0 , F_v and F_v/F_m were significantly affected by Cd concentration gradients. Increasing Cd levels in the soil caused a linear increase in F_0 , while both F_v and F_v/F_m decreased in low concentrations of Cd (about 0–100 mg/kg). This reducing trend stopped at higher levels of Cd in the soil at the range of 100 to 300 mg/kg (Figs. 5a, 5c, and 5d). However, increase in Cd levels in the soil had no significant effect on the F_m (Fig. 5b). The parameters F_0 and F_m are the levels of fluorescence at which Q_A is maximally oxidized (PSII centers open) and reduced (PSII centers closed), respectively. The F_v parameter shows the

ability of PSII to perform photochemistry (Q_A reduction) and F_v/F_m is the maximum efficiency at which light absorbed by PSII is used for reduction of Q_A . This parameter is used as a sensitive indicator of plant photosynthetic performance [12, 29]. Thus, a reduction of this parameter indicates the photoinhibitory or photo-oxidative effects of stress factors on PSII [7, 29]. In the present study, increase in F_0 and decrease in F_v and F_v/F_m , due to increased Cd level in the soil, are in agreement with previous reports by Li et al. [7] in *E. argyi*, Shahabivand et al. [9] in wheat, and Celeste Dias et al. [6] in lettuce.

In order to detect the relationship between effective photochemical quantum yield of PSII $Y(II)$, quantum yield of regulated energy dissipation $Y(NPQ)$, and quantum yield of non-regulated energy dissipation $Y(NO)$ with Cd concentration gradients, a segmented model was fitted separately to each data. The fitted models are presented in Fig. 5. As Cd level increased from 0 to 300 mg/kg, $Y(II)$ significantly decreased from 0.73 to 0.59 (20%), while $Y(NPQ)$ and $Y(NO)$ increased up to 54 and 48%, respectively (Figs. 5e–5g and Fig. 6). As shown in Figs. 5e–5g, the most variation in $Y(II)$, $Y(NPQ)$, and $Y(NO)$ was observed in the lower concentrations of Cd in the soil (in 0 to 75 mg/kg of Cd). Since, $Y(II)$ is the yield of photochemistry and directly related to the CO_2 assimilation rate [30], decreased $Y(II)$ in this study indicated that CO_2 assimilation was inhibited by increase in Cd concentration. The parameter $Y(NPQ)$ is the yield for dissipation by

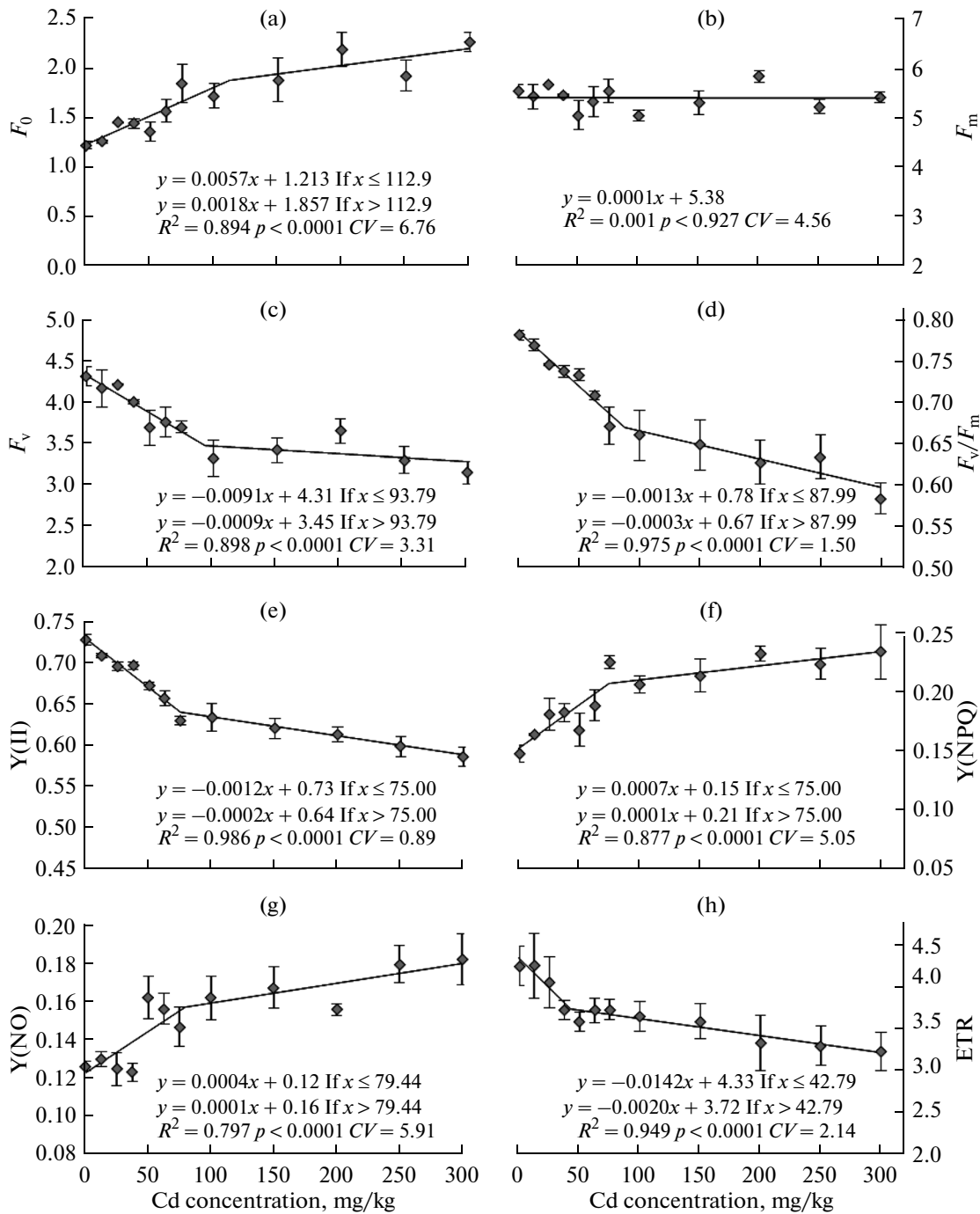


Fig. 5. The response of minimum (F_0) (a) and maximum (F_m) (b) fluorescence intensity, variable fluorescence (F_v) (c), maximum photochemical quantum yield of PSII (F_v/F_m) (d), effective photochemical quantum yield of PSII ($Y(II)$) (e), quantum yield of regulated energy dissipation ($Y(NPQ)$) (f), quantum yield of non-regulated energy dissipation ($Y(NO)$) (g), and electron transport rate (ETR) (h) to Cd concentrations in the soil using the segmented (eq. 10) and linear (eq. 11) models. Values are mean of three replicates and bars indicate \pm SE ($n = 3$).

down-regulation and $Y(NO)$ is the yield of other non-photochemical losses and reflects non-light induced quenching processes [30]. $Y(II)$, $Y(NPQ)$, and $Y(NO)$ are complementary and the sum of them is equal to 1 [13, 30].

Electron transport rate (ETR) significantly decreased following a segmented model when the Cd concentration in the soil increased up to 300 mg/kg (Fig. 5h). According to Maxwell and Johnson [29], under laboratory conditions, ETR highly correlated

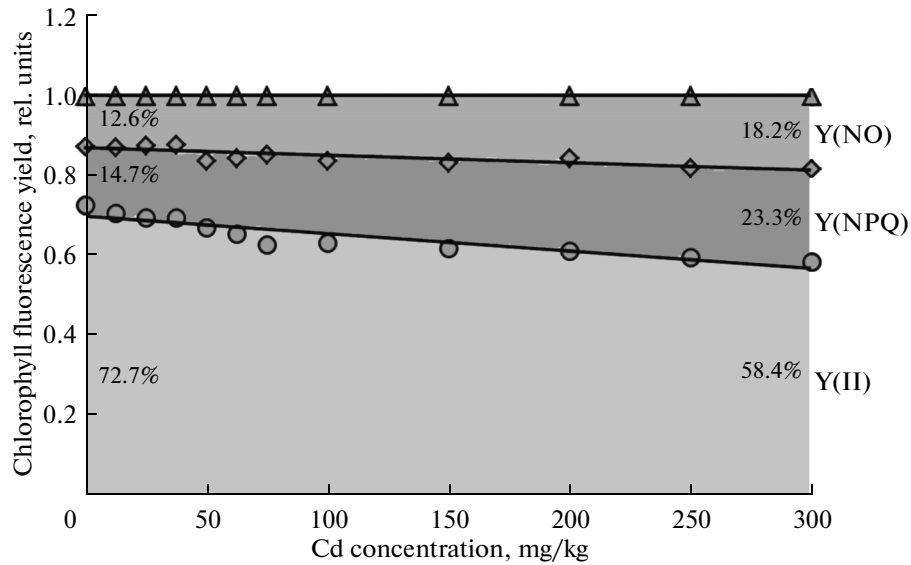


Fig. 6. Complementary changes of effective photochemical quantum yield of PSII (Y(II)), quantum yield of regulated energy dissipation (Y(NPQ)), and quantum yield of non-regulated energy dissipation (Y(NO)) in response to Cd concentrations in the soil.

with CO₂ assimilation rate. However, Flexas et al. [31] reported that there is a good agreement between CO₂ assimilation and ETR in C₄-plants, but not as good as in C₃-plants, due to the contribution of other processes to electron use. Therefore, in the present study, the reduction of purslane plants growth may be attributed to ETR reduction and limitation of CO₂ assimilation. Furthermore, Li et al. [7] reported that excess Cd in the soil could increase F_0 , F_m and reduce the values of F_v/F_m , Y(II), and ETR and thus, inhibit CO₂ assimilation in plants. The strongest variation in fluorescence parameters was observed in Y(NO) as 28.24 and 47.66% in 79.44 and 300 mg/kg of Cd in the soil, respectively (Fig. 5g).

In conclusion, the response of purslane plants growth related parameters and Chl *a* fluorescence attributes to Cd concentration gradients in the soil was quantified in this study. The influence of Cd gradients in the soil to all measured parameters was described either by a segmented (17 parameters) or a linear (two parameters) models. Growth characteristics, SPAD value, photosynthetic pigments content, and some parameters of Chl *a* fluorescence such as F_v , F_v/F_m , Y(II), and ETR decreased when Cd concentration increased. When F_0 , Y(NPQ), and Y(NO) increased, F_m was not affected significantly due to Cd level increase. In general, most variations in the studied parameters occurred in low concentrations of cadmium (approximately from 0 to 125 mg/kg) and then continued with less slope. The sensitivity of chlorophyll fluorescence parameters to Cd concentration gradients was lower than growth parameters and photosynthetic pigments. Moreover, among chlorophyll fluorescence parameters, Y(NPQ) was the most sensi-

tive to Cd concentration gradients in the soil, that can be due to disturbances of antennae complex of PSII.

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