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ORIGINAL PAPER

Cadmium toxicity affects photosynthesis and plant growth at different levels

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Abstract In this article we discuss and update some of the effects of Cd toxicity on the photosynthetic apparatus in a model crop *Lactuca sativa*. Seeds of *L. sativa* were germinated in solutions with 0, 1, 10 and 50 μM of $Cd(NO_3)_2$ and then transferred to a hydroponic culture medium. After 28 days, the effects of Cd on the photosynthetic apparatus of lettuce were analysed. Exposure of lettuce to 1 μM $Cd(NO_3)_2$ affected already plant growth (dry biomass), but, did not induce serious damages in the photosynthetic apparatus. However, increasing concentrations of this metal to 10 and 50 μM promoted a strong reduction of the maximum photochemical efficiency of PSII and an impairment of net CO_2 assimilation rate, putatively due to Rubisco activity decrease. This ultimately results in a strong inhibition of plant growth. Nutrient uptake and carbohydrate assimilation were also severely affected by Cd.

Keywords Cadmium · Nutrients · Photosynthesis · Pigments · Rubisco

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Abbreviations

P_N	Net CO_2 assimilation rate
ci/ca	Ratio of intercellular to atmospheric CO_2 concentration
Chl	Chlorophyll
DW	Dry weight
E	Transpiration rate
FW	Fresh weight
F_v/F_m	Maximal efficiency of PSII
g_s	Stomatal conductance
Rubisco	Ribulose 1,5 bisphosphate carboxylase/oxygenase
RWC	Relative water content

Introduction

Cadmium (Cd) is one of the most highly toxic trace pollutants for humans, animals and plants. Several studies demonstrated its cytotoxic, mutagenic and/or carcinogenic effects in animal cells. In plants, available data also suggest that Cd leads to cytotoxic and genotoxic effects (e.g. Santos et al. 2010).

Cd occurs naturally in soils (e.g. in complexes), but the anthropogenic emissions, mostly due to mining activities, burning of fossil fuels, metallurgical industry and the intensive use of phosphate fertilizers are the main sources of soil contamination (Singh and Agrawal 2007). Cadmium has relative mobility in soils and does not bind strongly to organic matter (Nelson and Campbell 1991). The degree to which plants are able to uptake Cd is conditioned by its concentration in the soil, and its bioavailability modulated by the presence of organic matter, pH, redox potential, temperature and concentrations of other elements (Benavides et al. 2005).

Cadmium stress in plants leads to a battery of stress symptoms that include chlorosis, necrotic lesions, wilting, disturbances in mineral nutrition and carbohydrate metabolism, and may therefore strongly reduce biomass production (e.g. Azevedo et al. 2005a; Santos et al. 2010). The photosynthetic apparatus is particularly susceptible to Cd and a reduction of photosynthesis is a common response in plants exposed to Cd (Burzynski and Klobu 2004).

The risk of Cd uptake by crops, followed by the transfer in the food chain, is an issue of high concern in nowadays. Lettuce is a worldwide important crop, one of the most consumed leafy vegetables in the human dietary (McBride 2003) and a high Cd-accumulating species (Monteiro et al. 2009). Moreover, a large part of this crop is grown in greenhouses, usually using special substrates and fertilization techniques involving reutilization of water, therefore implying an increased risk of heavy metal contamination (Gill et al. 2012). Despite the rising concern about metal pollution and further implication on plant reducing productivity, there are few studies regarding the effect of Cd on photosynthesis in lettuce: Costa and Morel (1994) applied Cd to plants after germination and found no significant effects on gas exchange; later, Monteiro et al. (2009) using high Cd concentrations found Cd-induced variations in the photosynthetic efficiency (F_v/F_m) and pigment contents. This comprehensive lack of knowledge supports that there is much still to be done in order to fully understand the extent of the effects of Cd in photosynthesis, and on nutrients involved in this metabolic process. Therefore, the objectives of this work are to understand how exposure to Cd concentrations that can be found in moderately and highly contaminated soils (Pál et al. 2006) leads to its accumulation in plant organs and how it affects photosynthesis in lettuce. For that we used a hydroponic system similar to many commercial horticultural productions, and analysed a large battery of photosynthetic parameters as well as the most relevant nutrients implied in the photosynthetic process.

Materials and methods

Plant material and culture conditions

Lactuca sativa L. (cv Reine de Mai) seeds were kept in dark for 4–5 days on moistened filter paper at three Cd concentrations [1, 10 and 50 μM of $Cd(NO_3)_2$] for germination. A group of seeds were germinated in the same conditions but only with distillated water (control). The germinated seedlings were transferred to an aerated hydroponic culture based on Hoagland's medium with 0, 1, 10 or 50 μM $Cd(NO_3)_2$. Cultures were maintained in a growth chamber at a temperature of 20 ± 2 °C, a 16/8 h (day/night) photoperiod with a photosynthetic photon flux density (PPFD) of app. 200 ± 20 μmol m^{-2} s^{-1}. The treatments were arranged in a randomized complete block design, and the experiment was performed two times. After 28 days from the beginning of Cd treatment, a full expanded leaf of each of the plant for each treatment was used for photosynthetic parameters measurements and then excised and used for measuring other parameters.

Determination of Cd content and nutritional status

Cd concentration in the hydroponic culture medium was routinely verified by inductively coupled plasma atomic emission spectroscopy (ICP-AES, JobinYvon, JY70 Plus, Longjumeau Cedex, France).

Root and leaf samples were immersed in 0.5 mM $CaSO_4$ for 10 min to remove (by cation exchange) Cd adsorbed to the tissue surface and rinsed in distilled water. Then, the leaf and root samples were dried for 48 h at 80 °C, weighted, ground to fine powder and treated as described by Azevedo et al. (2005a). Accumulation of Cd and the content of micronutrients, Fe and Mn, and macronutrients, Ca and Mg, were determined by ICP-AES.

Determination of plant growth, RWC and photosynthetic parameters

Roots and leaves dry weight and fresh weight were measured after 28 days of treatment. Dry weight was determined after drying the samples in an oven at 80 °C till constant weight.

The relative water content [$RWC = (FW-DW)/(TW-DW) \times 100$, where FW is the leaf fresh weight, DW is the leaf dry weight and TW is the leaf turgid weight (determined after floating the leaf samples for 180 min on distillate water at 5 °C in darkness)] was determined.

Gas exchange parameters, net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E) and the ratio of intercellular to atmospheric CO_2 concentration (c_i/c_a) were recorded on fully expanded leaves at 28 days after sowing using an infra-red gas analyzer (IRGA, LC*pro*+, ADC, Hoddesdon, United Kingdom). Measurements were always performed in the middle of the daily photoperiod at growth temperature (24 ± 2 °C) conditions and atmospheric CO_2 concentration.

Chlorophyll *a* fluorescence measurements were performed in situ in fully expanded leaves with a Plant Efficiency Analyser (Hansatech Instruments Ltd., UK) after 28 days of Cd exposure. Maximum photochemical efficiency of PSII was calculated as $F_v/F_m = (F_m-F_0)/F_m$ by measuring the fluorescence signal from a dark-adapted leaf when all reaction centres were open using a low intensity pulsed measuring light source (F_0) and during a pulse

saturating light (pulse of 3,000 μmol photons m^{-2} s^{-1} of white light) when all reaction centres were closed (F_m). Leaves were dark-adapted for 30 min using dark-adapting leaf-clips (FMS) for these measurements.

Quantification of chlorophylls and carotenoids

Leaf discs (0.2 g) were ground in a mortar to a powder in 2 ml cold acetone/Tris 50 mM pH 7.8 buffer solution (80:20, v:v) and centrifuged at 2,800×*g* during 5 min as described by Dias et al. (2012). The supernatant was diluted to a final volume of 3 ml with additional acetone/Tris buffer. The absorbance at 470, 537, 647 and 663 nm was determined with a Thermo Fisher Scientific spectrophotometer (Genesys 10-uv S). The contents of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids were calculated using the formulae of Sims and Gamon (2002).

Rubisco activity and protein content

Leaf samples (0.1 g) were ground in a mortar to a powder with liquid nitrogen and suspended with 1 ml of a specific buffer as described by Dias and Brüggemann (2007). Ribulose 1,5 bisphosphate carboxylase/oxygenase (Rubisco, EC 4.1.1.39) activity was extracted and assayed as described by Lilley and Walker (1974). This assay follows NADPH oxidation measured spectrophotometrically at 340 nm. Total activity was achieved after incubation in 20 mM $MgCl_2$ and 10 mM $NaHCO_3$ for 20 min. For protein determination, leaves (0.5 g) were homogenized in 5 ml of extraction buffer in a pre-chilled mortar and pestle with liquid nitrogen. The extraction buffer contained 0.1 M potassium phosphate buffer (pH 7.8), 5 mM Na_2 EDTA, 1 % PVP, 0.2 % Triton X-100, 1 mM PMSF and 2 mM DTT (Dias et al. 2011). The homogenate was centrifuged at 8,000×*g* for 20 min at 4 °C. The supernatant obtained was used for total soluble protein quantification. Soluble protein concentration was determined according to the method of Bradford (1976) using the Total Protein Kit, Micro (Sigma).

Soluble sugars and starch

Soluble sugars were extracted from leaf discs with 80 % (v/v) of ethanol at 80 °C over 20 min according to Correia et al. (2005). Glucose, fructose, sucrose and starch were quantified according to Correia et al. (2005) in a Thermo Fisher Scientific spectrophotometer (Genesys 10-uv S).

Data analyses

Data were analysed by one-way analysis of variance (ANOVA) using the Sigma Stat program for Windows, version 3.1. Comparisons between means were evaluated by a post hoc test (Holm-Sidak Test, Multiple Comparison Test) at a significant level set to 0.05.

Results

Cadmium concentration was below the ICP-AES detection limit in leaves and roots of control plants (Table 1). Data for Cd accumulation on lettuce tissues indicated that most of the metal was accumulated in roots and a lower fraction of this metal was translocated to the leaves (Table 1). Leaves and roots exposed to 10 and 50 μM Cd showed a significantly higher Cd accumulation than those under 1 μM Cd.

Micro and macronutrient contents in lettuce leaves and roots are presented in Table 2. In roots, only Mn uptake was affected showing a significant decrease at 10 and 50 μM Cd. Relative to leaves, Fe decreased significantly in plants exposed to 10 and 50 μM Cd; Mn uptake decrease was observed in leaves exposed to 1 and 50 μM Cd. Contrarily, Mg (a Rubisco co-factor) uptake increase significantly in plants exposed to 50 μM while Ca increase was observed in leaves exposed to 10 and 50 μM Cd.

After 28 days, lettuce plants presented a survival rate of 100 % growing at 1 and 10 μM Cd, and 97.7 % in 50 μM of Cd. The survival rate in control plants was 100 %.

Plant dry weight decreased significantly in plants exposed to Cd (Fig. 1a). Plants exposed to 1 and 10 μM Cd showed a reduction of 16 and 46 % of plant dry weight, respectively, compared to control plants. The strongest reduction in plant dry weight (76 %) was observed at the highest Cd concentration. The RWC was not affected by Cd exposure (Fig. 1b).

Lettuce plants exposed to 1 μM Cd showed similar P_N to control plants (Fig. 2a). However, a significant decrease (79 %) in P_N was observed in plants exposed to 10 μM Cd. As observed for the P_N, the g_s and E were also similar in control and in plants exposed to 1 μM Cd, but, in plants exposed to 10 μM Cd, g_s and E decreased significantly (Fig. 2b–c). A similar c_i/c_a was observed in control and in plants exposed to 1 μM Cd. However, in plants under 10 μM Cd, the c_i/c_a increased significantly (Fig. 2d). In lettuce plants exposed to 50 μM Cd, P_N was undetectable. Therefore, data from P_N, g_s, E and c_i/c_a for this concentration are not shown.

The analysis of chlorophyll *a* fluorescence showed that the basal fluorescence, F_0, was similar in control and in plants exposed to 1 μM Cd (Fig. 3a). However, in plants growing at 10 and 50 μM Cd a significant increase in F_0 was observed as compared to the control plants. A different pattern was observed for the F_m (Fig. 3b). Despite the similar values of F_m in control and in plants exposed to

Table 1 Cadmium content (mg g^{-1} DM) in leaves and roots of control and Cd-exposed lettuce plants

Cadmium content	Control	1 μM	10 μM	50 μM
Leaves	<D.L.a	0.012 ± 0.005b	0.123 ± 0.019c	0.679 ± 0.088d
Roots	<D.L.a	0.030 ± 0.003b	0.324 ± 0.047c	1.221 ± 0.048d

Different letters indicate significant differences between treatments at a significant level equal to 0.05. Results are means of six replications ±SD <D.L. means that the nutrient quantification by ICP-AES was below the detection limit

Table 2 Nutrients content (mg g^{-1} DM) in control and in Cd-exposed lettuce leaves and roots

Nutrient content	Control	1 μM	10 μM	50 μM
Roots				
Ca	7.722 ± 2.199a	10.122 ± 1.490a	6.711 ± 2.371a	7.454 ± 2.984a
Fe	0.251 ± 0.044a	0.211 ± 0.030a	0.261 ± 0.047a	0.255 ± 0.043a
Mg	2.485 ± 0.558a	2.581 ± 0.535a	2.208 ± 0.518a	2.547 ± 0.702a
Mn	0.212 ± 0.108a	0.195 ± 0.041a	0.056 ± 0.006b	0.046 ± 0.015b
Leaves				
Ca	13.690 ± 1.576a	14.189 ± 0.452a	17.722 ± 1.372b	19.975 ± 1.227b
Fe	0.136 ± 0.039a	0.097 ± 0.008ab	0.077 ± 0.006b	0.078 ± 0.011b
Mg	4.313 ± 0.393a	3.899 ± 0.151a	4.177 ± 0.162a	6.589 ± 0.329b
Mn	0.110 ± 0.013a	0.078 ± 0.005b	0.122 ± 0.007a	0.074 ± 0.007b

Results are means of three (roots) and five (leaves) replications ± SD. Different letters indicate significant differences between treatments at a significant level equal to 0.05. <D.L. means that the nutrient quantification by ICP-AES was below the detection limit

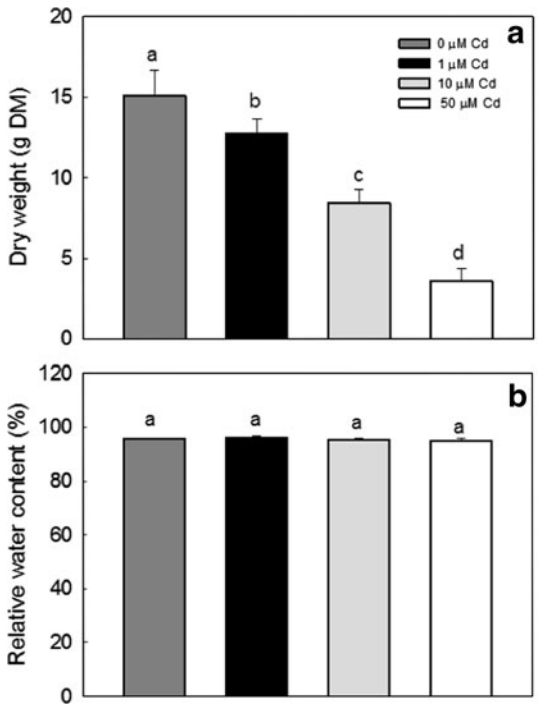

Fig. 1 Total biomass (**a**) and relative water content (**b**) in control and Cd-exposed lettuce plants. Results are means of six replications ± SD. *Different letters* indicate significant differences between treatments at a significant level equal to 0.05

1 μM Cd, a significant decrease was observed when lettuce plants were exposed to 10 and 50 μM Cd. The F_v/F_m decreased significantly with increasing Cd concentration in the culture medium to 10 and 50 μM (Fig. 3c). Control and plants exposed to 1 μM Cd showed the highest F_v/F_m values.

After 28 days of exposure, chlorosis was visibly more pronounced in leaves exposed to 10 and 50 μM Cd. Furthermore, accentuated necrosis and leaf fall were observed at the highest Cd concentration. These visual observations were consistent with the chlorophyll contents (Fig. 4). The exposure of lettuce to 10 and 50 μM Cd decreased significantly the concentration of Chl *a* and *b* relative to control plants (Fig. 4a–b). The ratio of Chl *a*/*b* decreased significantly in Cd-exposed plants. Plants exposed to 1 and 10 μM Cd showed a similar Chl *a*/*b* ($P > 0.05$) (Fig. 4c).

The concentration of carotenoids was similar in control and in plants exposed to 1 μM Cd (Fig. 4d). However, exposure of lettuce to 10 and 50 μM Cd induced a significant increase in carotenoids compared to control plants.

Rubisco maximal activity decreased significantly in lettuce leaves exposed to 10 and 50 μM Cd (Table 3). The strongest decrease in Rubisco activity was 95 % in plants exposed to 50 μM Cd followed by the 74 % in 10 μM Cd. Control and plants exposed to 1 μM Cd showed similar Rubisco activities.

Control and plants exposed to 1 μM Cd showed significantly higher soluble protein concentration than those exposed to 10 and 50 μM Cd (Table 3). A decrease of approximately 26 and 25 % of total soluble protein was observed in plants exposed to 10 and 50 μM Cd, respectively, compared to control plants.

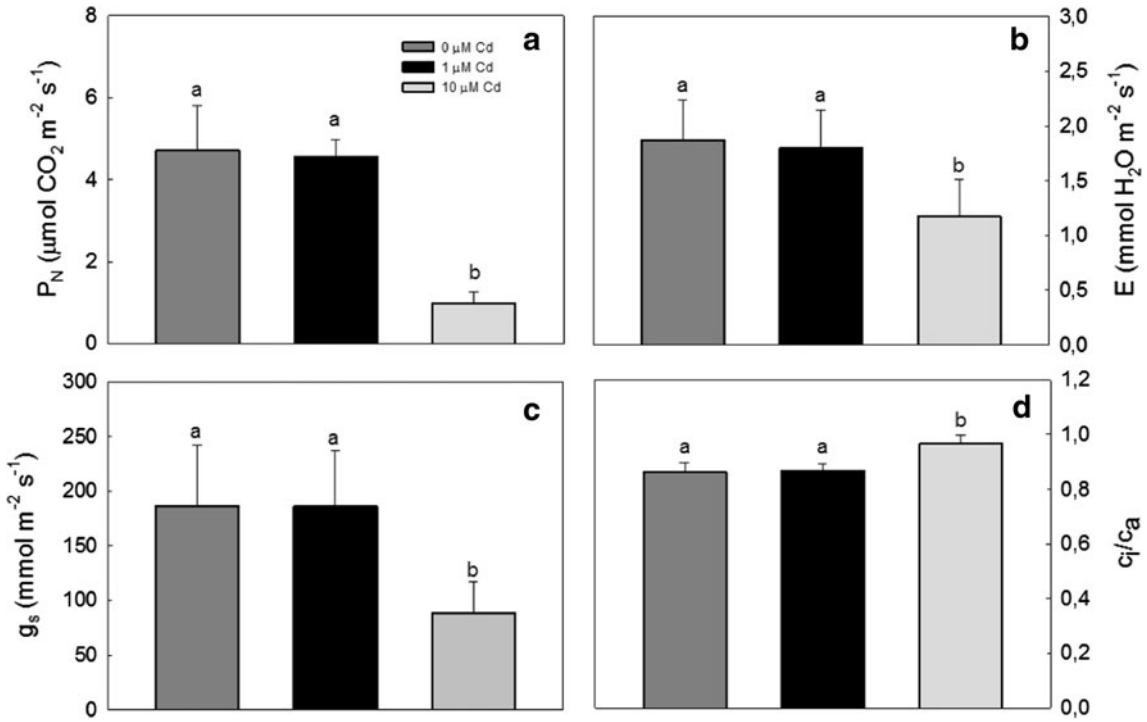

Fig. 2 Net photosynthetic rate (P_N) (**a**), transpiration rate (E) (**b**), stomatal conductance (g_s) (**c**), and intercellular to atmospheric CO_2 concentration ratio (c_i/c_a) (**d**) in control and Cd-exposed lettuce plants. Results are means of six replications ± SD. *Different letters* indicate significant differences between treatments at a significant level equal to 0.05

Glucose and fructose concentration increased significantly in lettuce plants exposed to 10 µM Cd and decreased significantly at the highest Cd concentration, relative to control plants (Table 4). Sucrose concentration increased significantly in lettuce leaves exposed to 10 and 50 µM Cd. No significant changes were observed in starch content between control and Cd-exposed leaves ($P < 0.05$) (Table 4).

Discussion

Cd is the most commonly found metal in soil which limits the crop productivity worldwide as this metal tend to accumulate within plant organs and negatively interfere with essential physiological processes (Gill et al. 2012). Lettuce exposure to Cd leads to an accumulation of Cd mainly in roots than in leaves, although the capacity to accumulate this metal depends on the Cd concentration in the culture medium. The results obtained are consistent with those reported for several species that demonstrated that Cd ions are mainly retained in the roots despite still considerable amounts are translocated to the shoots (in a ratio of approximately 1:2 in lettuce) (e.g. Wójcik and Tukiendorf 2005; Mobin and Khan 2007; López-Climent et al. 2011; Gill et al. 2012). Partitioning of metals in different plant parts is a common strategy to avoid toxicity in above-ground parts (López-Climent et al. 2011).

One of the crucial factors of Cd influence on plant metabolism and physiological processes is its relationship with other mineral nutrients (Dong et al. 2006). Cd uptake ions occurs via the same transmembrane carriers used to uptake Ca^{2+}, Fe^{2+}, Cu^{2+} and Mg^{2+} (Roth et al. 2006; Papoyan et al. 2007). In this work, lettuce exposure to Cd (10 and 50 µM) significantly decreased leaf uptake of several essential elements such as Fe and Mn. In roots, only in the highest concentration of Cd tested a significant reduction of Mn was noticed. These results suggest that Cd interferes with the translocation of macro- and micronutrients to the leaves.

Iron-deficiency is a recognized consequence of plant exposure to Cd and other metals, and has implications for several biological processes (Krupa et al. 1999). Apart from growth and chlorophyll synthesis, Cd-induced Fe deficiency affects the photosynthetic electron transport (Krupa et al. 1999). In agreement with this, the reduction of F_v/F_m may be associated to the decrease in leaf-Fe. These results are similar to those reported in lettuce by Monteiro et al. (2009) and also in other plant species (López-Millán et al. 2009). Manganese is an essential micronutrient with a very important role in the photolysis of H_2O by PSII and in the assimilation of NO_2^- in chloroplasts (Fodor 2002). The deficiency of Mn in lettuce roots and leaves might cause an impairment of such processes. Mn deficiency was reported by Lagriffoul et al. (1998) in other species exposed to Cd.

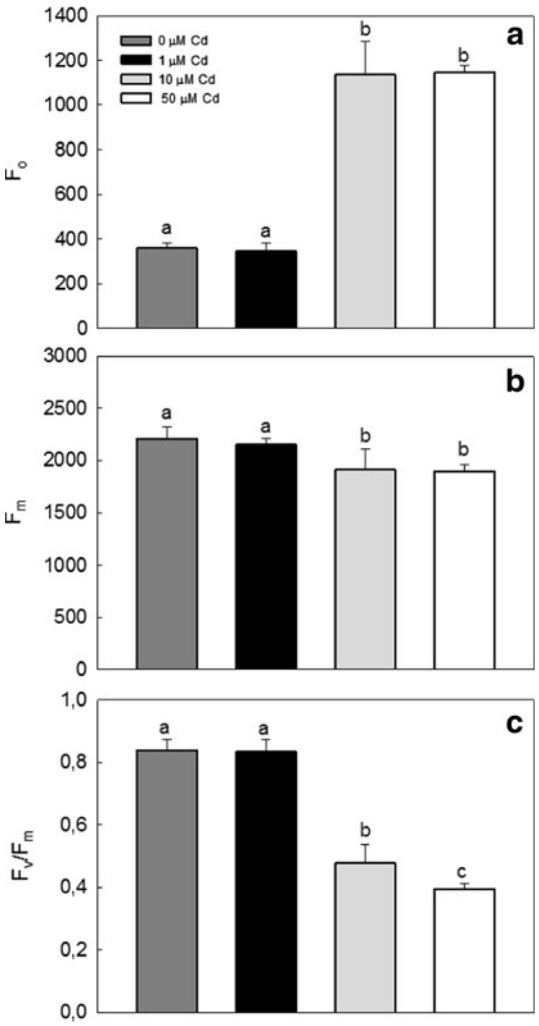

Fig. 3 Minimal (F_0) (**a**) and maximal (F_m) (**b**) chlorophyll fluorescence yield of dark-adapted leaves and maximal photochemical efficiency of PSII (F_v/F_m) (**c**) in control and Cd-exposed lettuce plants. Results are means of six replications ± SD. *Different letters* indicate significant differences between treatments at a significant level equal to 0.05

The accumulation of Cd in plant tissues caused damages to the photosynthetic apparatus. In particular, the results indicated that only for Cd concentrations higher than 10 µM, P_N is strongly inhibited. Also, this inhibition was followed by a decrease in E and g_s and an increase of the ratio of c_i/c_a. These results indicate that despite the stomatal closure observed, the increase in c_i/c_a supports that non-stomatal limitation strongly contributes to the reduction of the P_N. A limitation of CO_2 uptake by reduced Rubisco activity cannot be ruled out since a strong reduction of maximal Rubisco activity (74 % less than control plants) followed by a high protein loss (26 % less than control plants) was observed. Cd toxicity seems to be associated with a decrease in total protein content and inhibition of Rubisco activity (Siedlecka et al. 1997) and these changes are appointed to be one of the most important symptoms of Cd toxicity towards the photosynthetic apparatus (Krupa et al. 1999). Cd^{2+} ions interfere with Rubisco activation, lowering its activity and damaging its structure by substituting for Mg^{2+} ions, which are important cofactors of the carboxylation reactions, and may also shift Rubisco activity towards oxygenation reactions (Pietrini et al. 2003). Beside these effects, Cd stress is typically related to oxidative stress (Azevedo et al. 2005b) and a decrease in Rubisco activity can also be a result of oxidative activity (Wang et al. 2011). We have demonstrated recently for lettuce, using the same Cd concentrations, that Cd above 1 µM induced an increase of H_2O_2 while the total antioxidant capacity decreased, leading to increases in lipid and protein oxidation (Monteiro et al. 2012). Moreover, we demonstrated that all Cd concentrations induced some genotoxic effects (Monteiro et al. 2012).

The principal end products of photosynthesis are sugars. The strong reduction of the P_N at 10 µM Cd was accompanied by an increase of soluble sugar. This reflects a sugar utilization blockage by Cd as observed by the reduction in total biomass in Cd-exposed lettuce. Under stress conditions, soluble sugar increase can act as osmolyte to maintain leaf cell turgor (Rolland et al. 2006). Moreover, high sucrose contents are usually related to a sugar-mediated source-sink feedback inhibition of CO_2 assimilation or due to a reduction of sucrose hydrolysis due to changes in invertase activity (Podazza et al. 2006). Krapp and Stitt (1995) reported that decreased activity of Rubisco is often correlated with sugars accumulation in leaves and Roh and Choi (2004) demonstrated that the highest Rubisco activity was achieved when in vitro cultured tobacco plants were grown at 4 % sucrose, but, it was significantly reduced for higher sucrose concentrations (5 %). Taking these findings into consideration, the sucrose accumulation observed in the present work in lettuce leaves exposed to 10 and 50 µM Cd may be related to the reduction in Rubisco activity and concomitant decrease in P_N and plant growth.

Contrarily to the observed correlation between dry weight decrease and negative effects in the photosynthetic related parameters measured in plants exposed to 10 and 50 µM Cd, at the lowest Cd concentration (1 µM), plant dry weight decreased although, in general, the other parameters measured showed to be not affected by this low Cd concentration. Monteiro et al. (2012) showed for the same species under the same experimental conditions that 1 µM Cd did not induce oxidative stress (contrarily to 10 and 50 µM Cd) and that at this low concentration, only DNA breaks (genotoxicity) were demonstrated (Comet assay) together with a slight accumulation of root tip cells at G_2 phase. Considering our data and those of the literature, we hypothesize that while at high Cd doses, several cell parameters (photosynthesis, nutrient accumulation,

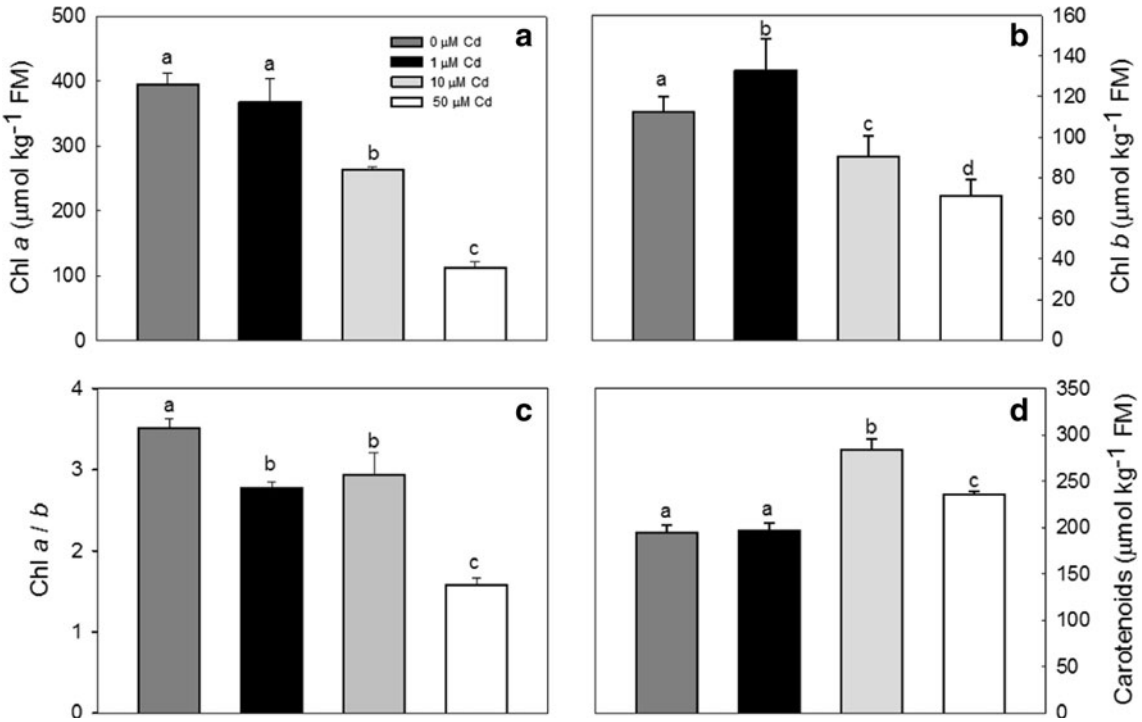

Fig. 4 Chl *b* (**a**), Chl *a* (**b**), Chl *a/b* (**c**) and carotenoids (**d**) concentrations in control and Cd-exposed lettuce plants. Results are means of six replications ± SD. *Different letters* indicate significant differences between treatments at a significant level equal to 0.05

Table 3 Rubisco in vitro maximum activity (µmol mg^{-1} FM s^{-1}) and soluble protein (mg g^{-1} FM) in control and Cd-exposed lettuce plants

	Control	1 µM	10 µM	50 µM
Rubisco activity	404.0 ± 60.9a	390.7 ± 42.1a	104.8 ± 27.8b	19.6 ± 0.65c
Soluble protein	10.1 ± 0.6a	10.8 ± 0.6a	7.5 ± 0.7b	7.6 ± 1.4b

Results are means of six replications ± SD. Different letters indicate significant differences between treatments at a significant level equal to 0.05

Table 4 Concentration of glucose, fructose, sucrose and starch (mmol g^{-1} FM) in control and Cd-exposed lettuce plants

	Control	1 µM	10 µM	50 µM
Glucose	144.3 ± 18.4a	148.9 ± 11.9a	287.0 ± 14.6b	79.7 ± 4.9c
Fructose	175.1 ± 45.3a	151.4 ± 15.3a	271.2 ± 16.2b	38.0 ± 5.8c
Sucrose	78.7 ± 3.9a	78.1 ± 2.3a	101.1 ± 7.4b	131.9 ± 8.3c
Starch	36.7 ± 5.7a	27.8 ± 3.9a	26.0 ± 3.9a	28.2 ± 6.9a

Results are means of six replications ± SD. Different letters indicate significant differences between treatments at a significant level equal to 0.05

oxidative stress, genotoxicity and cell progression) are negatively affected all contributing to reduced biomass (Azevedo et al. 2005a; Monteiro et al. 2009, 2012), at 1 µM Cd biomass reduction (dry weight decrease) may mostly be due to induced genotoxicity and delayed cell division (Monteiro et al. 2012).

The F_v/F_m characterizes the maximal efficiency of excitation energy capture by "open" PSII reaction centres and this parameter is usually used as a sensitive indicator of plant photosynthetic performance (Rod et al. 2012). Low Cd concentrations, 1 µM, had no negative effects on the F_v/F_m of lettuce plants. These values were similar to the control plants and are within the range expected for healthy plants (Dias et al. 2012). However, in lettuce plants exposed to 10 and 50 µM Cd, the increase of F_0 and decrease of F_m was resulted in a reduction of the F_v/F_m. An

increase of F_0 points to photo damage, whereas a decline in F_m reflects enhanced non-radiative energy. These results of F_v/F_m are typical of stressed plants and indicate reduced light-harvesting efficiency. The F_v/F_m was found to be reduced in several plant species, including lettuce, exposed to Cd (Azevedo et al. 2005a; Küpper et al. 2007; Bi et al. 2009; Monteiro et al. 2009; Kummerová et al. 2010).

Chlorophyll contents abruptly declined with increasing Cd concentrations, but, Cd affected more strongly Chl *a* than Chl *b*. Chl content was also strongly reduced in barley (Vassilev et al. 2002), tomato (Ammar et al. 2008; López-Millán et al. 2009), maize (Ekmekçi et al. 2008), mustard (Mobin and Khan 2007) and garden cress (Gill et al. 2012) exposed to Cd. Chlorosis might be due to the observed reduction of Fe in leaves and to the negative effects of Cd on chlorophyll metabolism (Chaffei et al. 2004). A putative degradation of chlorophyll and/or the inhibition of its biosynthesis were proposed by Sandalio et al. (2001) and have been reported as one of the causes for the impairment of photosynthesis and growth reduction produced by this metal.

Carotenoids act as light-harvesting pigments, and can protect chlorophyll and membranes destruction by quenching triplet chlorophyll and removing oxygen from the excited chlorophyll–oxygen complex (Young 1991). Carotenoids increase in lettuce plants exposed to 10 and 50 μM Cd may reflect an attempt to protect chlorophyll and/or the photosynthetic apparatus from the photooxidative destruction (Choudhury and Behera 2001) of Cd toxicity.

In conclusion, the present work demonstrated that even the lowest Cd concentration (1 μM) studied induced a reduction in plant growth (dry biomass). Moreover, germination and further growth of lettuce at high Cd levels (10 and 50 μM) induces severe impairment of photosynthesis (undetectable P_N for the case of 50 μM Cd) leading ultimately to a reduction in plant productivity by a strong reduction in plant growth.

Author contributions M.C. Dias and C. Santos planned the experiments, made data analysis and wrote the manuscript. J. Moutinho-Pereira, C. Correia and B. Gonçalves measured the gas exchange. M.C. Dias and C. Monteiro performed the growth exposures, Cd content, mineral content, RWC, dry weight, Chl *a* fluorescence, pigment content, Rubisco activity, protein content and carbohydrate content.

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