Plant Physiology and Biochemistry 106 (2016) 30–38

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Physiological and biochemical mechanisms preventing Cd-toxicity in the hyperaccumulator *Atriplex halimus* L.

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ARTICLE INFO

Article history:
Received 24 February 2016
Received in revised form
20 April 2016
Accepted 22 April 2016
Available online 23 April 2016

Keywords:
Antioxidative enzyme activity
Fluorescence
Growth parameters
Mineral nutrients
Photoinhibition
Photosynthesis

ABSTRACT

The xero-halophyte *Atriplex halimus* L., recently described as Cd-hyperaccumulator, was examined to determine Cd toxicity threshold and the physiological mechanisms involved in Cd tolerance. An experiment was conducted to investigate the effect of cadmium from 0 to 1350 μM on chlorophyll fluorescence parameters, gas exchange, photosynthetic pigment concentrations and antioxidative enzyme activities of *A. halimus*. Cadmium, calcium, iron, manganese, magnesium, potassium, phosphorous, sodium and zinc concentrations were also analyzed. Plants of *A. halimus* were not able to survive at 1350 μM Cd and the upper tolerance limit was recorded at 650 μM Cd; although chlorosis was observed from 200 μM Cd. Cadmium accumulation increased with increase in Cd supply, reaching maxima of 0.77 and 4.65 mg g^{-1} dry weight in shoots and roots, respectively, at 650 μM Cd. Dry mass, shoot length, specific leaf area, relative growth rate, net photosynthetic rate, stomatal conductance, pigments contents and chlorophyll fluorescence were significantly reduced by increasing Cd concentration. However, the activities of superoxide dismutase (SOD; EC1.15.1.1), catalase (CAT; EC1.11.1.6) and guaiacol peroxidase (GPx; EC1.11.1.7) were significantly induced by Cd. Exposures to Cd caused also a significant decrease in P contents in roots, Mg and Mn contents in shoots and Fe and K contents in roots and shoots and had no effect on Ca, Na and Zn contents. The tolerance of *A. halimus* to Cd stress might be related with its capacity to avoid the translocation of great amounts of Cd in its aboveground tissues and higher activities of enzymatic antioxidants in the leaf.

1. Introduction

Heavy metal pollution has become an important environmental problem today and increasing continuously as a result of growing industrialization and the massive use of fertilizers. Cadmium is one of the most phytotoxic elements, because of the high water solubility, relative mobility and long biological half-life (Wang et al., 2014). It is not an essential nutrient in higher plants, and the exposure to relatively low concentrations results in high toxicity to plant and animal (Redondo-Gómez et al., 2010). Several studies have shown that plant metabolism is affected by Cd in different ways, and the photosynthetic process appears to be particularly sensitive to this metal (Ci et al., 2010). In particular, Cd causes chlorosis and growth reduction (Zemanová et al., 2015). Cd can interact with the plant water balance (Costa and Morel, 1994), disturb nutrient uptake (Redondo-Gómez et al., 2010), inhibits stomatal opening (Perfus-Barbeoch et al., 2002), provokes damages to the photosystems I and II (Küpper et al., 2007; Li et al., 2015), and inhibits some of the enzymes of the Calvin cycle (Kabata-Pendias and Pendias, 2001). High concentration of Cd induces increased respiration and activities of tricarboxylic acid cycle as well as other pathways of carbohydrate utilization (Liphadzi and Kirkham, 2005).

Abbreviations: A_N, net photosynthetic rate; CAT, catalase; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; C_i, intercellular CO_2 concentration; *Cx* + *c*, carotenoids; GPx, guaiacol peroxidase; F_0, minimal fluorescence level in the dark-adapted state; F_m, maximal fluorescence level in the dark-adapted state; F_s, steady state fluorescence yield; F_v, variable fluorescence level in the dark-adapted state; F_v/F_m, maximum quantum efficiency of PSII photochemistry; Φ_{PSII}; actual quantum yield of PSII photochemistry, g_s; stomatal conductance, Φ_{NPQ}, quantum yield of non-photochemical quenching; ROS, reactive oxygen species; RGR, relative growth rate; SOD, superoxide dismutase; SLA, specific leaf area; iWUE, intrinsic water use efficiency.

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http://dx.doi.org/10.1016/j.plaphy.2016.04.041
0981-9428/

It has also been reported that Cd can increase the production of reactive oxygen species (ROS) in plants cells, which can cause peroxidative damages, such as cell membrane lipids peroxidation and protein carbonylation (Gill et al., 2012). To cope with oxidative damage, plants possess an efficient ROS scavenging system composed of enzymatic and non-enzymatic antioxidants. Among antioxidative enzymes, superoxide dismutase (SOD), guaiacol peroxidase (GPx) and catalase (CAT) play an important role in controlling the level of ROS (Qiu et al., 2008).

Atriplex halimus L. is a xero-halophyte which is perennial and native in arid and semi-arid Mediterranean regions. This species, that is present as a natural invading shrub in several mining areas of northern Africa and southern Europe (Pérez-Esteban et al., 2013), is known to grow rapidly and densely on degraded soils and tolerates extreme environmental conditions such as drought (Martínez et al., 2005), salinity (Bajji et al., 1998) and light stress (Streb et al., 1997). Recent work with *A. halimus* showed that it is hypertolerant to high concentrations of Cd up to 400 μM (Lefèvre et al., 2010; Nedjimi and Daoud, 2009) and Pérez-Esteban et al. (2013) described it as appropriate species for the phytostabilization of metals in mine soils. However, no studies are available concerning the Cd toxicity threshold in *A. halimus* and its physiological and biochemical mechanisms to prevent Cd toxicity. Although it is important to identify interesting hypertolerant species for phytoremediation, it is equally important to know the mechanisms underlying tolerance of these species. Therefore, this work is aimed to: (1) elucidate the Cd phytotoxicity thresholds in *A. halimus* by examining its growth response to different Cd levels; (2) determine the effect of Cd on the fluorescence parameters, gas exchange characteristics, anti-oxidative enzymes system (CAT, GPx and SOD) and photosynthetic pigments; and (3) examine the effect of Cd on nutrient status. Results obtained from this study may be useful for understanding the mechanisms of Cd tolerance in *A. halimus*.

2. Materials and methods

2.1. Plant material and Cd treatments

Seeds of *A. halimux* were collected in November 2013 from wild population grow at the arid salty area El-Outaya located in the province of Biskra, southeast of Algeria (34°55′42″N 5°38′58″E, and 198 m elevation). Seeds were removed from the bracts then stored at room temperature until use.

Seeds were placed in a germinator at 12 °C and photoperiod of 16 h of light for a month. The seedlings were then transferred separately into perlite filled pots (one plant per pot) and placed in a glasshouse with controlled temperature of 21–25 °C, 40–60% relative humidity and natural daylight (maximum light flux: 1000 μmol m^{-2} s^{-1}). Pots were irrigated with 20% Hoagland nutrient solution (pH 6.2) (Hoagland and Arnon, 1950).

After 70 days of seedling culture, the plants (n = 7) were exposed to six Cd treatments: 0, 50, 200, 400, 650 and 1350 μM Cd (supplied as $CdCl_2 \cdot 5/2H_2O$). Each treatment was allocated in one tray, each containing seven pots. During the treatment, the nutrient solution level in the trays was controlled every two days, and if necessary, 20% of Hoagland's solution was added to keep the level constant. The entire nutrient solutions were replaced every week to prevent nutrient and metal depletion. Cd concentrations were selected to cover variations recorded by Pérez-Sirvent et al. (2008) and Redondo-Gómez et al. (2009) in the salt marshes of southern Iberian Peninsula, where *A. halimus* is widely distributed. All measurements were carried out after 22 day of Cd treatment.

2.2. Sample collection and growth analysis

At the end of the experiment, plants (n = 7) were harvested, divided into shoots and roots and rinsed with distilled water to remove any perlite particles attached to plant surfaces. Growth parameters (shoots lengths, shoots and root dry mass) were measured. Specific leaf area (SLA) was calculated as leaf area of the sampled leaves divided by their dry mass. The dry mass was determined after drying shoots and roots at 80 °C for 48 h.

The relative growth rate (RGR) of whole plants was calculated as: RGR = (DMf - DMi) × D^{-1} (gg^{-1} day^{-1}), where DMf = final dry mass, DMi = initial dry mass and D = duration of experiment (days).

The symptoms of Cd toxicity (leaf senescence, and chlorosis) were registered by visual observation during the experiment. At the end of treatment, plant survival was recorded: plant was considered as dead if all leaves were not green.

2.3. Gas exchange and chlorophyll fluorescence analysis

Gas exchange and chlorophyll fluorescence measurements were carried out on fully expanded leaves before harvesting (n = 7, one measurement per plant).

Net photosynthetic rate (A_N), intercellular CO_2 concentration (C_i) and stomatal conductance (g_s) were determined according Mateos-Naranjo et al. (2013) using an open infrared gas analyzer system (Li-6400-40, Li-COR Inc., Lincoln, NE, USA). Intrinsic water use efficiency (iWUE) was calculated as the ratio between A_N and gs.

Chlorophyll fluorescence measurements were made using a portable modulated fluorimeter (FMS-2, Hansatech Instruments Ltd., UK). Light- and dark-adapted fluorescence were taken at dawn (stable, 50 μmol m^{-2} s^{-1} ambient light) and at midday (1600 μmol m^{-2} s^{-1}) as described by Mateos-Naranjo et al. (2013) to investigate the effect of Cd stress on the sensitivity of plants to photoinhibition.

Using fluorescence parameters determined in both light- and dark-adapted states, the following were calculated: actual quantum yield of PSII photochemistry [$\Phi_{PSII} = (F_m' - F_s)/F_m'$] (Genty et al., 1989) and quantum yield of non-photochemical quenching, which is the regulatory light-induced non-photochemical quenching [$\Phi_{NPQ} = (F_s/F_m') - (F_s/F_m)$] (Lazár, 2015). Φ_{PSII} relates to achieved efficiency in a plant under a given treatment and indicates the proportion of absorbed energy being used in photochemistry, while Φ_{NPQ} provides an indication of the amount of energy that is dissipated in the form of heat (Maxwell and Johnson, 2000).

Chronic (PI_{chr}) and dynamic (PI_{dyn}) photoinhibition were calculated according to Werner et al. (2002) as:

$$PI_{chr} = \left[(F_v/F_m)_{max} - (F_v/F_m)_d\right](F_v/F_m)_{max} \times 100$$

$$PI_{dyn} = \left[(F_v/F_m)_d - (F_v/F_m)_{mid}\right](F_v/F_m)_{max} \times 100$$

where $(F_v/F_m)_d$ and $(F_v/F_m)_{mid}$ are dawn and midday F_v/F_m values, respectively. $(F_v/F_m)_{max}$ is the maximum F_v/F_m value, which was calculated as the average of dawn measurements of the control one day after imposing Cd treatments.

2.4. Photosynthetic pigments

Photosynthetic pigments of five shoots per treatment were extracted using 0.1 g of fresh material in 5 ml of 80% aqueous acetone. After centrifuging, 1 ml of the suspension was diluted with a further 2 ml of acetone and chlorophyll *a* (Chl *a*), chlorophyll *b*

(Chl *b*) and carotenoid (C*x* + *c*) contents were determined using three wavelengths (663.2, 646.8 and 470.0 nm). Concentrations of pigments (μg g fw^{-1}) were obtained through calculation (Lichtenthaler, 1987).

2.5. Antioxidant enzyme analysis

At the end of experiment, 500 mg of fresh leaf tissues were powdered in liquid nitrogen then homogenized under cold conditions in 8.0 ml of extraction buffer containing 50 mM phosphate buffer (pH 7.6) with 0.1 mM EDTA. The homogenate was centrifuged at 8923 rpm for 20 min at 4 °C. The supernatant was used in enzymatic analysis, and the specific enzyme activities were expressed as units per μg of protein. The analysis for protein content was carried out according to Bradford (1976), using bovine serum albumin as a standard.

Catalase (CAT; EC1.11.1.6) was assayed according to the protocol of Teranishi et al. (2014) in a mixture of 890 μL of sodium phosphate buffer (50 mM, pH 7.0), 100 μL of leaf extract and 10 μL H_2O_2 (15%). Enzyme activity was calculated as the decrease in absorbance at 240 nm using the molar extinction coefficient for H_2O_2 (39.4 mM^{-1} cm^{-1}). One unit of the enzyme is defined as the amount necessary to decompose 50% H_2O_2 for 60 s. Guaiacol peroxidase (GPx; EC1.11.1.7) was determined according to Zhou et al. (1997) in 1 ml of reaction mixture containing 590 μL of sodium phosphate buffer (50 mM, pH 7.0), 200 μL of H_2O_2 (50 mM) and 10 μL of leave extract. The reaction was initiated with the addition of 200 μL of guaiacol (20 mM). Increase in absorbance is monitored for 2 min at 470 nm and one enzyme activity unit is defined as the amount of enzyme that catalyzes the conversion of one μmole of H_2O_2 in 60 s (guaiacol molar extinction coefficient $\varepsilon = 26.6\ mM^{-1}\ cm^{-1}$). Superoxide dismutase (SOD; EC1.15.1.1) was assayed according to Marklund and Marklund (1974) by calculating the inhibition of pyrogallol autooxidation by SOD at 325 nm for 2 min. Reaction mixture (1 ml) contained 550 μL sodium phosphate buffer (50 mM, pH 7.6), 360 μL Milli-Q water and 10 μL of plant extract. The reaction was started with the addition of 80 μL Pyrogallol (3 mM). One enzyme activity is defined as the amount of enzyme capable of inhibiting 50% of the autooxidation of pyrogallol.

2.6. Chemical analysis of plant samples

For determination of Cd and mineral nutrient in roots and shoots, dried samples were ground to a fine powder. Samples, of 0.5 g each, were then digested with 6 ml HNO_3, 0.5 ml HF and 1 ml H_2O_2. The contents of Cd, Ca, Fe, K, Mn, Mg, K, P, Na and Zn in the extract were measured by inductively coupled plasma (ICP) spectroscopy (ARL-Fison 3410, USA).

Translocation factor (TF) and bioaccumulation factor (BF) were calculated as follows (Chen et al., 2011):

$$TF = [Cd]shoot/[Cd]root,$$

$$BF = [Cd]shoot/[Cd]solution,$$

2.7. Statistical analysis

Statistical analysis was performed using SPSS 19.0 statistical program (SPSS Inc., Chicago, IL, USA). Pearson coefficients were calculated to evaluate correlation between different variables. Data were analyzed using one- and two-way analysis of variance (ANOVA). Tukey's test was applied to establish the significance between different treatments ($P < 0.05$).

3. Results

3.1. Growth and survival

After 22 days of Cd treatment, no plants was able to survive at 1350 μM Cd, thus physiological measurements and chemicals analysis were not performed.

Cd influenced negatively the plant growth, therefore, above- and belowground dry mass and shoot length decreased with increasing Cd concentration ($r = -0.53$, -0.48 and -0.54, respectively, $P < 0.01$ for all three parameters, Fig. 1A–C). RGR and SLA also decreased significantly (about 71% for both parameters) with external Cd concentration ($r = -0.55$, $r = -0.67$, $P < 0.01$, Fig. 1B–D); and these reductions were due to the decrease on leaves number (data not shown).

Finally, 28% of the plants treated with 400 and 650 μM Cd were unable to survive after 22 days of treatment, and chlorosis was observed in *A. halimus* leaves from 200 μM Cd, and became more pronounced with the elevation in Cd levels.

3.2. Gas exchange

Plants grown at 650 μM Cd showed photosynthetic parameters outside the detection range of the infrared gas analyzer, and therefore they have not been represented in (Fig. 2A–D). Net photosynthetic rate (A_N) was highly decreased with increasing Cd concentration after 22 days of treatment ($r = -0.76$, $P < 0.01$, Fig. 2A). Furthermore, there was a positive relationship between A_N and RGR ($r = 0.65$, $P < 0.01$).

Stomatal conductance (g_s) followed a similar pattern as A_N and decreased with increasing Cd concentration ($r = -0.63$, $P < 0.01$, Fig. 2B). Moreover, no significant difference was observed for iWUE and C_i with Cd concentration (ANOVA, $p > 0.05$). However, C_i tended to be increased with Cd concentration ($r = 0.41$, $P < 0.01$, Fig. 2C), while iWUE tended to be decreased ($r = -0.40$, $P < 0.01$, Fig. 2D).

3.3. Photosynthetic pigments

Cd treatment caused a significant reduction in pigment concentrations (Chl *a*, Chl *b* and C*x* + *c*, all in μg g fw^{-1}) at 50 μM Cd (ANOVA, $P < 0.05$, Fig. 3A–C). Compared to the control, the reductions at 50 μM Cd were 60, 55 and 59% for Chl *a*, Chl *b* and C*x* + *c*, respectively.

3.4. Chlorophyll fluorescence

Values of F_v/F_m showed a slight but significant decline at midday and at dawn with the elevation in Cd levels (midday: $r = -0.48$, $P < 0.01$, Fig. 3, A; dawn: $r = -0.47$, $P < 0.01$; Fig. 4A).

The actual quantum yield of PSII photochemistry (Φ_{PSII}) showed the same pattern as F_v/F_m at dawn ($r = -0.51$, $P < 0.01$; Fig. 4B); compared to the control, the reduction in Φ_{PSII} was 24% at 650 μM Cd. At midday, Φ_{PSII} values decreased significantly above 50 μM Cd ($P < 0.05$), but showed no response to further increases in Cd concentration. $F_v/F_{m,}$ and Φ_{PSII} values were significantly lower at midday than at dawn in all treatments. The Φ_{NPQ} did not show any differences with external Cd concentration at dawn, whereas a significant increase in Cd toxicity on this parameter was observed at midday (midday: $r = 0.47$, $P < 0.01$, Fig. 4C).

Finally, the percentage of both chronic and dynamic photoinhibitions progressively increased with increasing Cd levels, reaching the maximum at 650 μM Cd (Fig. 5).

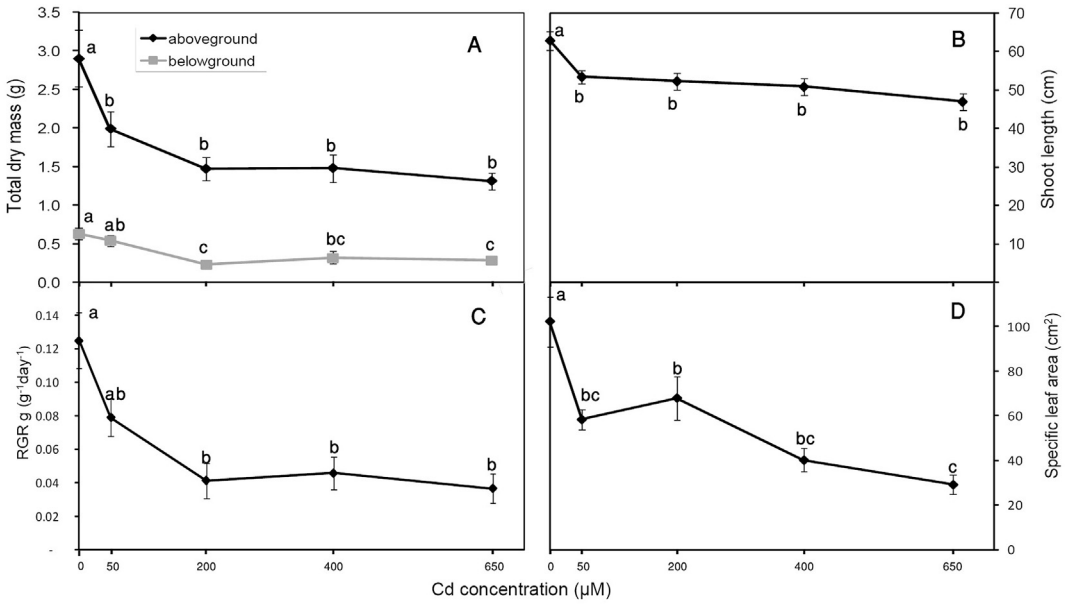

Fig. 1. Total dry mass (above- and belowground biomass) (A), relative growth rate, RGR (B), specific leaf area (C) and shoots length (D) of *Atriplex halimus* L. after 22 d of treatment with different Cd levels. Each value represent mean of seven replicates ± SE. For each parameter, different letters indicate that means are significantly different (Tukey test, $P < 0.05$).

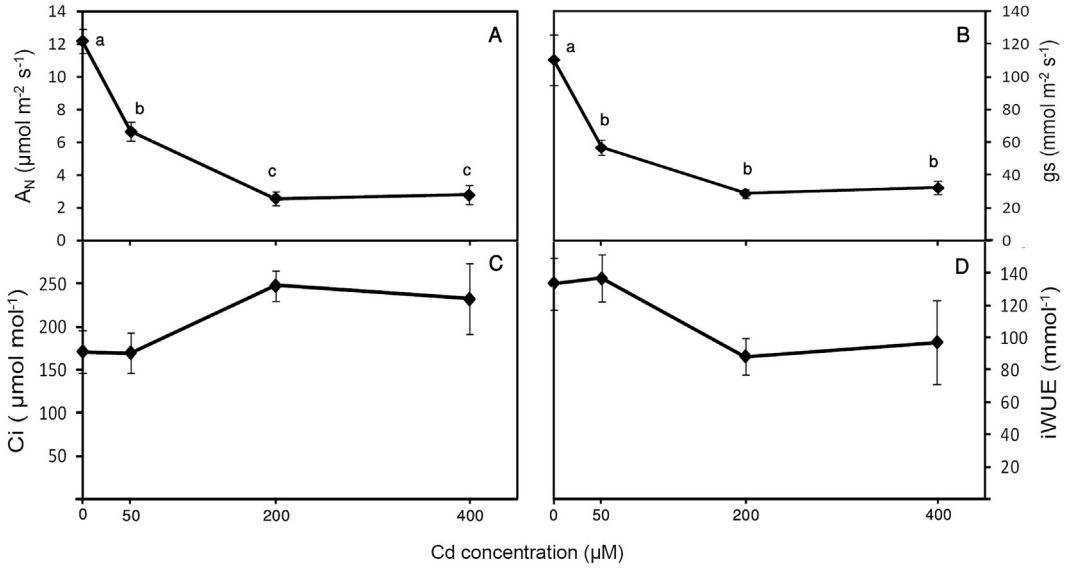

Fig. 2. (A) Net photosynthetic rate (A_N), (B) stomatal conductance (g_s), (C) intercellular CO_2 concentration (C_i) and (D) intrinsic water use efficiency (iWUE) in *Atriplex halimus* L. after 22 d of treatment with different Cd levels. Each value represent mean of seven replicates ± SE. For each parameter, different letters indicate that means are significantly different (Tukey test, $P < 0.05$).

3.5. Antioxidative enzymes

The antioxidant enzyme activities of GPx, CAT and SOD were significantly enhanced by Cd concentration (Fig. 6). GPx activity increased significantly reaching a maximum at 400 µM Cd, then decreased at 650 µM Cd (Fig. 6A), but remained significantly higher than control ($p < 0.05$). CAT and SOD activities showed the same trends as GPx (Fig. 6B–C).

3.6. Chemical analysis of plant samples

The Cd concentration both in roots and shoots increased significantly, and correlates directly with the Cd concentration of the medium ($r = 0.97$, $P < 0.0001$; $r = 0.96$, $P < 0.0001$, for root and shoot, respectively; Fig. 7A). Cd accumulation was higher in roots than in shoots (two-way ANOVA, $P < 0.0001$), thus, (TF) was always lower than 1 (Table 1). Furthermore, (BF) was higher than one in all treatments, but it decreased significantly with Cd concentration (Table 1).

Shoot P, root Mn, and root and shoot Mg concentrations showed no significant overall response to Cd concentration (Fig. 7). Contrary, root P, shoot Mn and root and shoot Fe and K decreased significantly with increasing external Cd concentration ($p < 0.05$). In absence of cadmium, Fe concentrations was about 3- and 7-fold higher in shoots and roots, respectively (ANOVA, $P < 0.001$; Fig. 7E). Otherwise, K, Mn and Mg concentrations were higher in shoot than

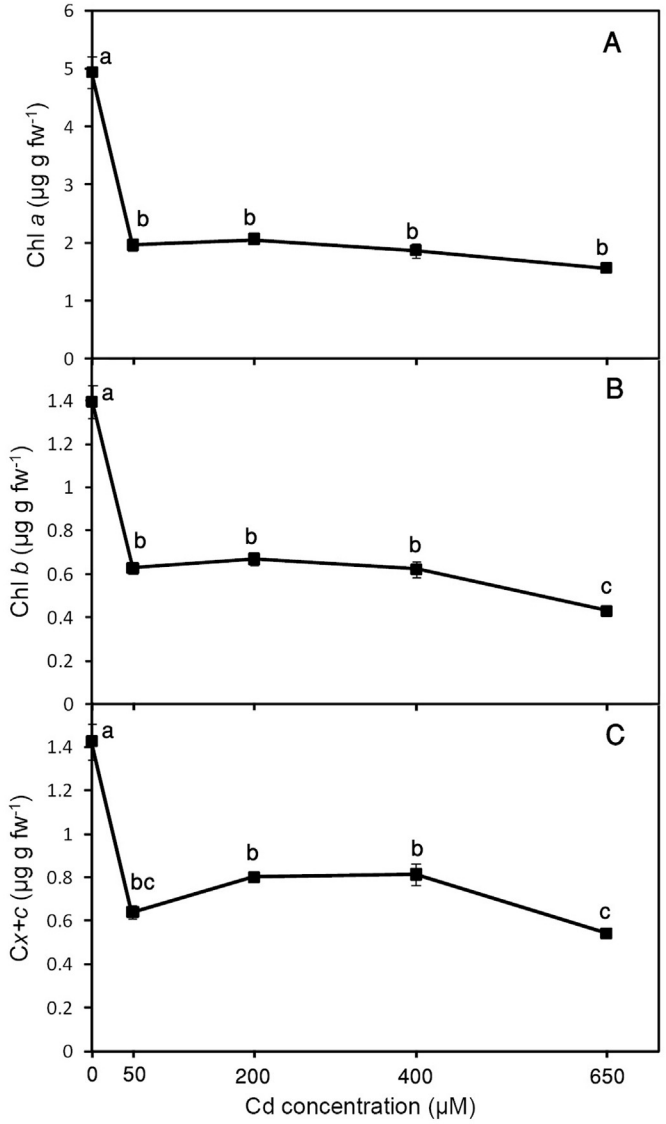

Fig. 3. (A) Chlorophyll *a* (Chl *a*), (B) chlorophyll *b* (Chl *b*) and (C) carotenoid (C*x* + *c*) concentrations in *Atriplex halimus* L. after 22 d of treatment with different Cd levels. Each value represent mean of five replicates ± SE. For each parameter, different small letters indicate that means are significantly different (Tukey test, P < 0.05).

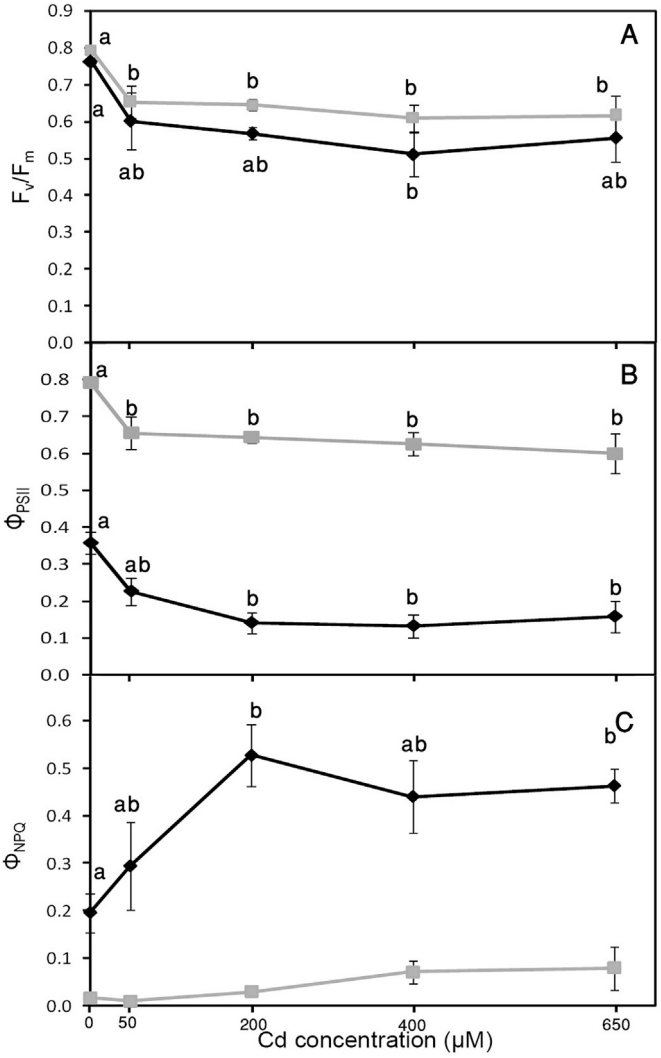

Fig. 4. (A) Maximum quantum efficiency of PSII photochemistry (F_v/F_m), (B) actual quantum yield of PSII photochemistry (Φ_{PSII}) and (C) quantum yield of non-photochemical quenching (Φ_{NPQ}) at midday (◆) and at dawn (■) in *Atriplex halimus* L. after 22 d of treatment with different Cd levels. Each value represent mean of seven replicates ± SE. For each parameter, different letters indicate that means are significantly different (Tukey test, P < 0.05).

in roots for all treatments; while P and Fe tissue concentrations were higher in roots than in shoots, except for K at 650 µM Cd. Finally, Ca, Na and Zn in both root and shoot were not influenced by Cd treatment (data not shown).

4. Discussion

We have studied the Cd toxicity threshold in the xero-halophyte *Atriplex halimus* and its physiological mechanisms to prevent Cd toxicity. For this we analyzed the response of growth, survival, photosynthetic apparatus, antioxidative enzymes system and mineral composition of this species to Cd stress. Our results showed that the accumulation of Cd was much higher in roots than in shoots in all treatments, which is in agreement with Nedjimi and Daoud (2009) who studied *A. halimus* subsp. *Schweinfurthii*. However, concerning the Cd uptake, we found about three folds higher in shoots and four folds higher in roots compared with Nedjimi and Daoud (2009) findings. This difference in the accumulation might

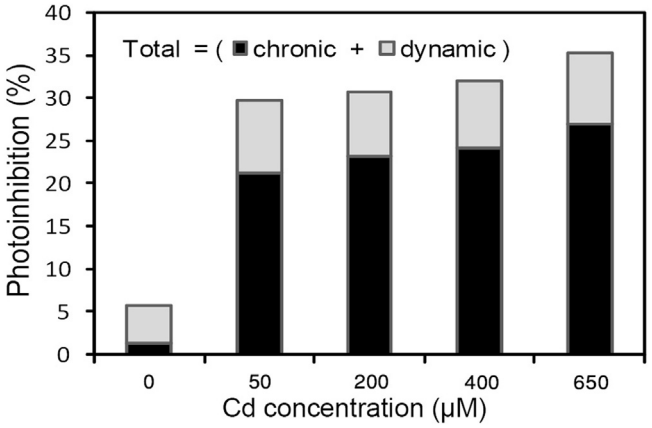

Fig. 5. Percentage of Chronic and dynamic photoinhibition in *Atriplex halimus* L. after 22 d of treatment with different Cd levels.

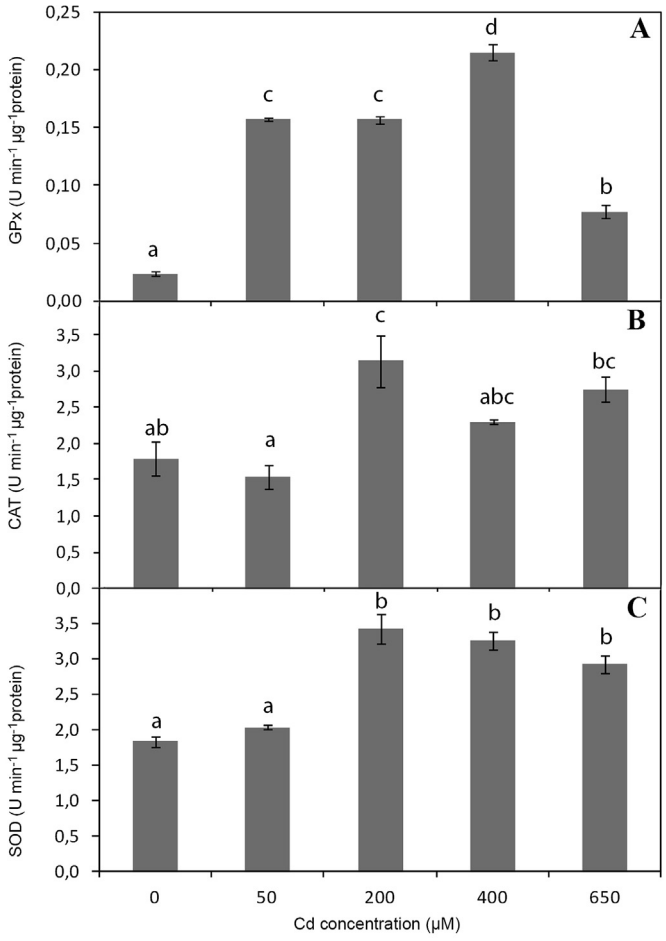

Fig. 6. (A) Guaiacol peroxidase (GPx), (B) catalase (CAT) and (C) superoxide dismutase (SOD) activities in leaves of *Atriplex halimus* L. after 22 d of treatment with different Cd levels. Each value represent mean of three replicates ± SE. For each parameter, different small letters indicate that means are significantly different (Tukey test, $P < 0.05$).

be caused by different growing conditions; Nedjimi and Daoud (2009) carried out their experiments with 35 day-old plants (grown in vermiculite), while we have used 70 day-old plants (grown in perlite). Anyway, *A. halimus* demonstrates the ability to accumulate Cd. Thus, bioaccumulation factor which used to assess the efficiency of metal accumulation (Redondo-Gómez et al., 2010), exceeded the critical value (1.0) under different Cd levels. However, Translocation factor, which should be taken into account while evaluating hyperaccumulators was lower than (1.0) for all Cd treatments, suggesting that *A. halimus* have low capacity to move Cd from root to shoot. According to Liu et al. (2009), plants with high bioaccumulation factor and low translocation factor have potential for phytostabilization.

In our study, Cd influenced negatively the growth of *A. halimus* plants in terms of dry weight, shoots length, RGR and SLA which is in accordance with the previous findings (Nedjimi and Daoud, 2009; Redondo-Gómez et al., 2010). The phytotoxicity threshold (PT50, tissue concentration corresponding to 50% reduction in growth) was between 420 and 650 mg kg^{-1} and between 1100 and 2660 mg kg^{-1}, for shoots and roots of *A. halimus*, respectively. This value is much higher than that reported by Nedjimi and Daoud (2009) which found that PT50 was 120 and 500 mg g^{-1} for shoots and roots, respectively. This difference may be linked to both the different state of development of the plants used in both experiments as well as the high genetic variability of this species (Abbad et al., 2004). On the other hand, our results concerning the plants survival indicate that LC50 (LC50, the lethal concentration that kills 50% of plants) was between 650 and 1350 µM Cd because all plants were died at 1350 µM Cd after 22 days. However, since A_N was inhibited entirely at 650 µM Cd, it can be conclude that this concentration is the upper tolerance limit of *A. halimus* under Cd stress.

Otherwise, reduced growth rate at high Cd concentrations can be attributed to the decrease recorded in the net photosynthetic rate. The results of our study showed that Cd treatments clearly decrease A_N and g_s values. Such a decrease in A_N and g_s under Cd stress has been recorded in many other species (Redondo-Gómez et al., 2010; Ying et al., 2010). The decline of A_N may be ascribed to stomatal (g_s) and/or non-stomatal limitations (Flexas and Medrano, 2002). Closing of the stomatal pores and/or decreasing in its density are known to reduce both A_N and C_i in plants (Cornic, 2000; Vitória et al., 2003; Zhu et al., 2005). However, in our experiment, C_i seemed to increase with increasing Cd concentration, suggesting that also a not stomatal restriction led to the decrease of A_N; which might be due to the decreased pigment content (Chen et al., 2011), as well as the inhibition of key enzyme activities in the Calvin cycle, photosynthetic electron transport chain and RUBISCO activity (Feng et al., 2010). In this way, we recorded a significant decrease in photosynthetic pigments concentration with the increase of Cd in the growth medium. Cd treatment has been shown to impair the structure of chloroplasts, the chlorophyll biosynthesis pathway and, even, the proper assembly of the pigment–protein complexes of photosystems (Li et al., 2008; Ouzounidou et al., 1997; Ridvan Sivaci et al., 2004; Wang et al., 2014). On the other hand, we recorded an overall reduction in iWUE; and Nedjimi and Daoud (2009) found a decrease in root hydraulic conductivity of *A. halimus* under Cd exposure. Poschenrieder et al. (1989) explained that the high levels of heavy metals can induce secondary water stress in plants.

The maximum quantum efficiency of PSII (F_v/F_m) is usually used as stress indicator (including Cd stress), and decrease in this parameter in an important sign of photoinhibition. Photoinhibition may cause a reduction in the normal photosynthesis rate and damage in the photosynthetic apparatus (Mateos-Naranjo et al., 2013). In our study, (F_v/F_m) and (Φ_{PSII}) were affected by Cd treatments at midday, suggesting that Cd excess enhances photoinhibition induced by light stress. The reduction in F_v/F_m values at midday indicated that *A. halimus* experienced dynamic photoinhibition at the higher light flux. This photoinhibition was caused by a lower proportion of open reaction centres (lower values of F_m) as a consequence of a saturation of photosynthesis by light. Also, Φ_{PSII} decreased as a result of the increase in Φ_{NPQ}, which suggests that the plants dissipated absorbed energy as heat (Wang et al., 2014). Maxwell and Johnson (2000) reported that increased thermal dissipation can be considered as photoprotective mechanism that preserves photosynthetic apparatus against oxidative damage. Photoinhibition is caused by damage to photosynthetic components, and this effect can be short (dynamic photoinhibition) and/or long reversible (chronic photoinhibition) (Werner et al., 2002).

Cd toxicity in plants is known to induce the generation of ROS, such as superoxide radicals (O^{2-}) and hydrogen peroxide (H_2O_2), which cause oxidative damage. The antioxidative enzymes such as GPx, CAT and SOD play an important role in controlling the level of ROS in plant (Qiu et al., 2008; Dazy et al., 2009; Parlak and Yilmaz, 2013). SOD can convert superoxide radicals to H_2O_2, while GPx and CAT can catalyze decomposition of H_2O_2 into H_2O (Dazy et al., 2009; Qiu et al., 2008). The increased antioxidant enzyme activities observed in this study may be related to an increase in the rate of ROS production as well as to the de novo synthesis of enzyme

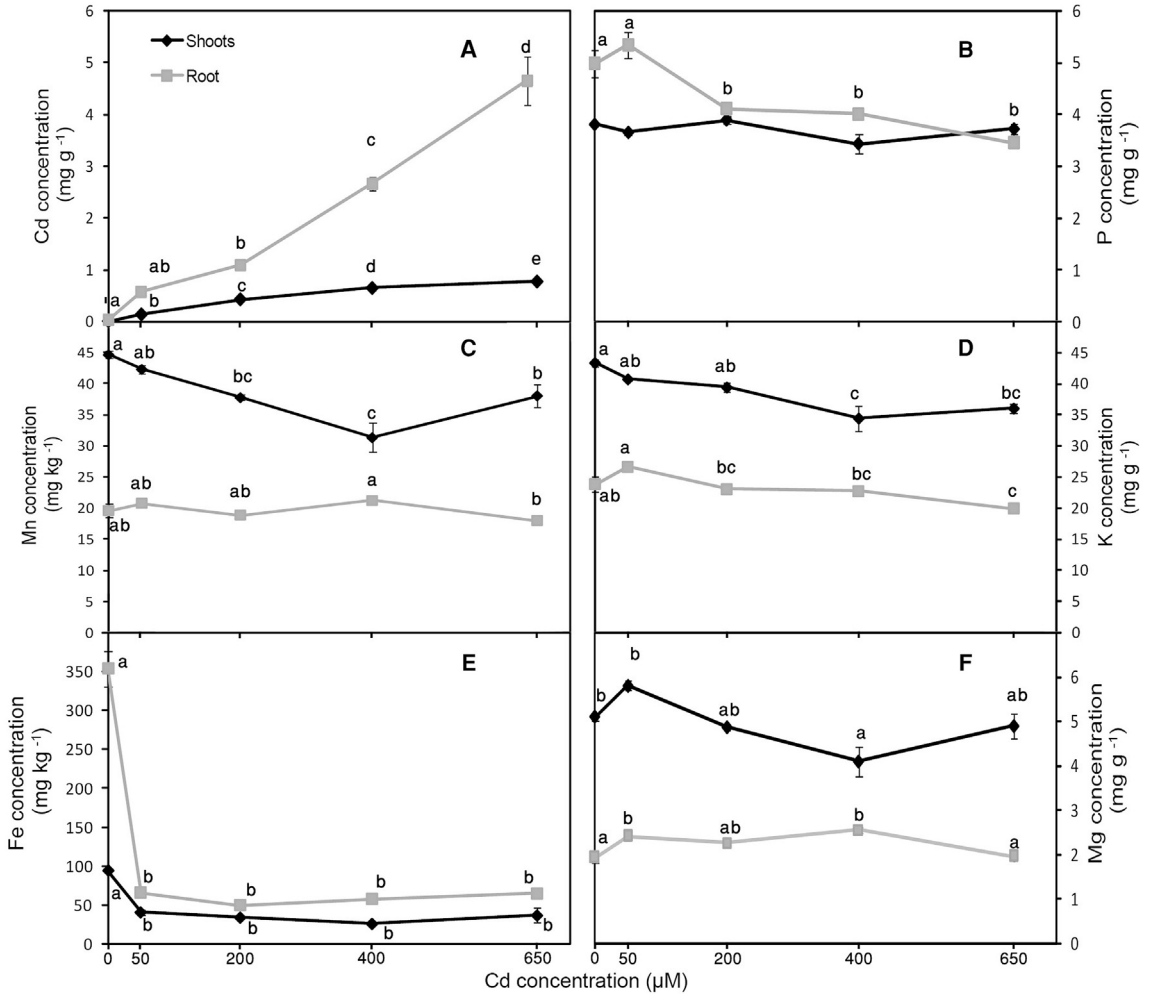

Fig. 7. Concentration of (A) cadmium, (B) phosphorous, (C) manganese, (D) potassium, (E) iron and (F) magnesium in shoots (◆) and roots (■) dry masses of *Atriplex halimus* L. after 22 d of treatment with different Cd levels. Each value represent mean of three replicates ± SE. For each parameter, different letters indicate that means are significantly different (Tukey test, $P < 0.05$).

Table 1
Effect of Cd on translocation and bioaccumulation factors of *A. halimus* L. after 22 d of treatment with different Cd levels. Each value represent mean of three replicates ± SE. For each parameter, different letters in the same line indicate that means are significantly different (Tukey test, $P < 0.05$).

	Cd concentration µM			
	50	200	400	650
Translocation factor	0.24 ± 0.004 ab	0.39 ± 0.01 c	0.25 ± 0.02 b	0.17 ± 0.02 a
Bioaccumulation factor	13.49 ± 0.21 a	10.6 ± 0.09 b	9.22 ± 0.53 c	6.42 ± 0.11 d

protein (Ruiz-Lozano et al., 1996). Moreover, the reduction in GPx activity observed in this study at the highest Cd concentration is probably due to their inactivation by accumulation of H_2O_2 (Singh et al., 2006).

The reduction in the absorption of essential mineral elements has been described as one of the effects of heavy metals on plants (Kabata-Pendias and Pendias, 2001). In this regard, we noted that Cd stress caused a considerable reduction in macronutrients (K, P and Mg) and micronutrients (Fe and Mn) in the shoots and/or roots tissues of *A. halimus* plants. Disturbances of mineral nutrition under Cd stress have been reported previously by a number of authors in several plant species like *Brassica napus* oleifera, *Triticum aestivum* (Zembala et al., 2010), *Fagus sylvatica* L. (Breckle and Kahle, 1992) and *Prunus dulcis* (Nada et al., 2007). According to Dong et al. (2006), Cd may interfere with nutrient uptake, by altering the plasma membrane permeability, and by affecting element transport processes across the membrane, particularly those with the same valence such as Fe and Mn. Küpper and Kochian (2010) suggest that the reduction in micronutrients like Mn was probably due to competition for transporters or interference with the expression of the transporter gene.

5. Conclusion

Exposure of *A. halimus* to Cd considerably affected its growth expressed as dry mass, shoots length, SLA and RGR and severely decreased it photosynthetic apparatus activity. According to plants survival and A_N values, the upper tolerance Cd threshold of *A. halimus* is close to 650 µM, although chlorosis was observed from 200 µM Cd. Cd excess affected also the photochemical (PSII)

apparatus, the photosynthetic pigment concentrations, the water relations and the absorption of essential mineral elements. Furthermore, limiting translocation of Cd from roots to shoots and enhancing photoinhibition and induction of antioxidant enzyme activities might contribute to improve tolerance of *A. halimus* to Cd stress. Finally, TF and BF values confirm that *A. halimus* could be useful in phytostabilization rather than phytoextraction; considering this species is consumed by beasts, this makes it more interesting for phytoremediation of low and moderate Cd-contaminated sites.

Author contributions

MM, EMN, BL and SRG conceived and designed the experiments. MM and SRG wrote the manuscript. MM, EMN and JMBP made ecophysiological measurements. MM, JMBP and JAPR determined antioxidative enzymes activities.

Acknowledgements

We acknowledge the staff from the University of Seville greenhouse for facilities during the experiment. We also acknowledge the Algeria Ministry of Higher Education and Scientific Research.

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