Photosynthetic pigments content, δ-aminolevulinic acid dehydratase and acid phosphatase activities and mineral nutrients concentration in cadmium-exposed Cucumis sativus L.

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Abstract: In this study, the effects of cadmium chloride (CdCl₂) on plant growth, histology of roots, photosynthetic pigments content, δ-aminolevulinic acid dehydratase (ALA-D; E.C. 4.2.1.24) and acid phosphatase activities (AP; E.C. 3.1.3.2), soluble phosphorus (Pi) measurement and mineral nutrients content in cucumber seedlings (Cucumis sativus L.) were investigated. Cucumber seedlings were grown in vitro in an agar-solidified substrate containing four CdCl₂ treatments (0, 100, 400, and 1000 µM) for ten days. Cd was readily absorbed by seedlings and its content was greater in the roots than in the shoot. Cd reduced shoot and root length, and fresh and dry biomass of seedlings. Inhibition of root cell elongation in Cd-treated seedlings was observed by the increase of the mean radial size of cells belonging to three zones of the root tip. The highest level of Cd reduced in a similar manner chlorophyll a, chlorophyll b and total chlorophyll contents. Increasing concentrations of Cd resulted in a linear decrease in carotenoids levels of cotyledons. Interestingly, the ALA-D activity in cotyledons was inhibited only at the highest level of Cd. Root and shoot AP activities were, respectively, activated and inhibited at all CdCl₂ concentrations. Root Pi concentration was increased in all Cd treatments and it was not altered in the shoot tissues. Moreover, in general, the nutrient contents were increased in the root and decreased in the shoot. Therefore, we suggest that Cd affects negatively growth, photosynthetic pigments, ALA-D and AP activities and partition of mineral nutrients in cucumber seedlings.

Key words: Cucumis sativus; ALA-D; cadmium; mineral nutrients; phosphatases; photosynthetic pigments

Introduction

Cadmium (Cd) is a heavy metal released into the environment by natural sources such as volcanoes, continental dusts or by weathering of the underlying bedrock. However, anthropogenic activities like mining production, use and disposal of batteries, metal contaminated wastes and sludge disposal, application of pesticides and phosphate fertilizers contribute to dispersion of Cd leading to environmental pollution (Alloway 1995). Moreover, Cd is easily absorbed by plant roots and translocated to above-ground tissues and then poses a potential threat to human health when it enters in the food chain.

Heavy metals like Cd have been shown to affect a wide range of plant cellular activities including photosynthesis, respiration and mineral nutrition leading to chlorosis and plant growth inhibition (Prasad & Prasad 1987; Ouzounidou et al. 1997; Sanità Di Toppi & Gabrielli 1999; Carnielutti et al. 2006). Cd can interfere on the photosynthesis by affecting the synthesis of photosynthetic pigments like chlorophyll and carotenoids and...
Cadmium-exposed *Cucumis sativus*

thus having an important role in the chloroplast development in young leaves (Sanitá Di Toppi & Gabbrilei 1999). In view of this, it becomes important to study the enzyme δ-aminolevulinic acid dehydratase (ALA-D), a sulphydryl-containing enzyme that catalyzes the asymmetric condensation of 2 molecules of δ-aminolevulinic acid (ALA) to porphobilinogen (Gibson et al. 1955) because this reaction is fundamental for the biosynthesis of tetrapyrroles such as porphyrins, hemes, and chlorophylls (Jaffe et al. 2000). Moreover, it is known that accumulation of ALA in the chlorophyll and heme pathway due to ALA-D inhibition or by deregulation of ALA synthesis and its consequent generation of reactive oxygen species (ROS) is indeed highly responsible for the deleterious action of Cd in soybean (Noriega et al. 2007).

Besides those alterations, Cd interacts with the metabolism of essential elements affecting nutrient uptake and distribution, resulting in mineral nutrient imbalance and physiological disorders (Jalil et al. 1994; Zhang et al. 2002). Therefore, it becomes crucial to study enzymes like acid phosphatases (APs) that catalyze the hydrolysis of inorganic phosphorus (Pi) from a broad range of phosphate monoesters and anhydrides (Vincent et al. 1992; Duff et al. 1994). This reaction is an important process in metabolic regulation, energy metabolism, and some cellular signal transduction routes of plant cells and is related with the maintenance of the phosphorus status of the plant (Duff et al. 1994). Also, phosphorus (P) plays a vital role in energy transfer and is an important macromolecular constituent, such as in phospholipids, proteins and nucleic acids (Vincent et al. 1992).

Cucumber is an important crop plant which was selected as a test plant species due to its sensitivity to a wide range of contaminants (Pereira et al. 2006; Tabaldi et al. 2007) and also to the insufficient information available on Cd toxicity in this species. In order to get more information on the mechanisms involved in the plant tolerance to this metal, we verified the effects of Cd on plant growth, histology of the roots, chlorophyll a, chlorophyll b, total chlorophyll and carotenoids contents, δ-aminolevulinic acid dehydratase and acid phosphatase activities, soluble phosphorus (Pi) and Ca, K, Mg, Fe, Zn, and Na contents.

**Material and methods**

**Plant material and growth conditions**

Cucumber seeds (*Cucumis sativus* L.) provided by Feltrin Ltd. (Santa Maria, RS) were germinated, and after the radicle had broken through, the seedlings were grown for 10 days in a growth chamber (16h light/dark) under light intensity of 35 µmol m⁻² s⁻¹ at plant level with a temperature of 25 ± 1°C. Cucumber seeds were kept in a glass recipient with 15 mL of medium containing CdCl₂ diluted in a 0.5% agar solution. The solution containing agar was heated and the cadmium solution was then added. No nutritive solution was added to the agar. The seedlings made use of the seed nutrition in the initial stage of development, and it had been verified, in a previous experiment, that up to the tenth day the plants did not suffer any nutrient deficiency (data not shown). Four CdCl₂ treatments (0, 100, 400, and 1000 µM) were applied randomly with 15 replicates and 6 seeds per pot.

**Growth analysis**

Cucumber growth was determined by measuring the length of the root system (Tennant 1975) and of the shoot (measured with a ruler). To obtain the fresh weight, excess water was removed with a paper towel after root washing. To obtain dry weight, the plants were left at 65°C to a constant weight.

**Metal determination**

Approximately 0.05 g of roots and shoot were digested with 4 mL HNO₃ utilizing the following stages of heating: a) 50°C for 1 h; b) 80°C for 1 h; and 120°C for 1 h in a digester block (Velp, Italy). The samples were then diluted to 50 mL with high-purity water. Cd concentrations were determined using a Model AAS 5 EA atomic absorption spectrometer (Analytic Jena, Germany) equipped with a transversely heated graphite furnace and an autosampler (MPB 5).

**Anatomical studies**

Root tip samples (3 mm length) were immediately fixed in glutaraldehyde solution for at least 24 h at room temperature. The samples were dehydrated in graded ethanol (30, 50, 70, 90, and 100%; for 1 h each), and embedded in Leica histo resin (AOTEC – Instrumentos Científicos Ltda.). Thin sections (5 µm) were successively taken until reaching 350 µm for cross and longitudinal sections, stained with toluidine blue and finally, optically examined and photographed with an Olympus PM C35B camera mounted on an Olympus CH30 microscope at a magnification of 400×. Permanent slides of cross sections (n = 6) of at least 3 different root tips at positions 100, 200 and 300 µm from the root cap were chosen to determine the mean radial diameter of cells. An adjustable micrometer scale mounted on the microscope eyepiece was used to measure the radial cell length by means of i) the precise measurement of the whole diameter of the root (taken at two different positions) ii) divided by the number of cells in that path.

**Chlorophyll and carotenoids contents**

Chlorophyll and carotenoids were extracted following the method of (Hiscox & Israelstam 1979). Briefly, 0.1 g of cotyledons was placed in a vial containing 7 mL dimethyl sulphoxide (DMSO). The photosynthetic pigments were extracted from the fluid without grinding at 65°C by incubating for 2 h. To liquid extract was added 3 mL of DMSO. A 3 mL sample was transferred to a cuvette and the absorbances at 645, 663 and 470 nm were read in order to determine chlorophyll a, chlorophyll b and carotenoids contents, respectively. Chlorophyll content was calculated following the equation used by (Arnon 1949).

δ-aminolevulinic acid dehydratase (ALA-D; EC 4.2.1.24) activity

Since the cotyledons presented high chlorophyll contents, they were used in the determination of ALA-D activity. Cucumber cotyledons were homogenized in 10 mM Tris-HCl buffer, pH 9.0, at a proportion of 1:1 (w/v). The homogenate was centrifuged at 12,000 g at 4°C for 10 min to yield a supernatant (S1) that was used for the enzyme assay. The supernatant was pre-treated with 0.1% Triton X-100 and 0.5 mM dithiothreitol (DTT). ALA-D activity...
was assayed as described by (Barbosa et al. 1998) by measuring the rate of porphobilinogen (PBG) formation. The incubation mixture for the assays contained 100 mM Tris-HCl buffer, pH 9.0 and 3.6 mM ALA. Incubation was started by adding 100 µL of the tissue preparation to a final volume of 400 µL and stopped by adding 350 µL of the mixture containing 10% trichloroactic acid (TCA) and 10 mM HgCl₂. The product of the reaction was determined with the Ehrlich reagent at 555 nm using a molar absorption coefficient of 6.1×10⁴ M⁻¹cm⁻¹ (Sassa 1982) for the Ehrlich-porphobilinogen salt.

**Acid phosphatases assay (AP, E.C. 3.1.3.2)**

Cucumber seedlings were separated into root and shoot and then were centrifuged at 43,200 g for 30 min and the resulting supernatant was used for enzyme assay. Acid phosphatase activity was determined according to (Tabaldi et al. 2007) in a reaction medium consisting of 3.5 mM sodium azide, 2.5 mM calcium chloride, 100 mM citrate buffer, pH 5.5 at a final volume of 200 µL. A 20 µL aliquot of the enzyme preparation was added to the reaction mixture, except in controls, and pre-incubated for 10 min at 35°C. The reaction was started by the addition of substrate and stopped by the addition of 200 µL of 10% TCA to a final concentration of 5%. Inorganic phosphate (Pi) was measured at 630 nm using malachite green as the colorimetric reagent and KHP₄ as standard for the calibration curve. All assays were performed using PPI as substrate at a final concentration of 3.0 mM.

**Soluble phosphorus content (Ps)**

Cucumber seedlings were separated into root and shoot and homogenized in a solution containing 100 mM Tris-HCl buffer (pH 7.4) and 0.1 mM EDTA, at a proportion of 1:20 (w/v). The homogenate was centrifuged at 8,000 g for 4°C for 10 min to yield a supernatant which was used to determine soluble phosphorus. An aliquot of the diluted sample (800 µL) was incubated at 45°C for 20 min in a medium containing 2.5 N sulfuric acid, 4.8 mM ammonium molybdate and 35 mM ascorbic acid in a total volume of 1 mL. A standard curve was constructed using K₂HPO₄. After cooling at room temperature the samples were read at 650 nm.

**Mineral nutrients content**

The cucumber seedlings were separated into shoot and roots and then were oven-dried at 65°C to constant weights. The plant material was ground with a stainless steel grinder and then it was digested in a mixture of HNO₃ – HClO₄ at a proportion of 4:1 (v/v). Potassium (K⁺) and sodium (Na⁺) concentrations were measured with a B262 flame spectrophotometer (Micronal, São Paulo, SP, Brazil). Calcium (Ca²⁺), iron (Fe²⁺), magnesium (Mg²⁺), and zinc (Zn²⁺) concentrations were measured with a GBC 932AAS atomic absorption spectrophotometer (GBC Scientific Equipment Pty Ltd, Victoria, Australia).

**Protein determination**

In all the enzyme preparations, protein was measured by the Coomassie Blue method according to (Bradford 1976) using bovine serum albumin as standard.

**Statistical analysis**

The experiments were done as randomized design. The analyses of variance were computed on statistically significant differences determined based on the appropriate F-tests.

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### Table 1. Cd accumulation in root and shoot of 10-day-old cucumber seedlings.

<table>
<thead>
<tr>
<th>CdCl₂ concentrations (µM)</th>
<th>Cd content (µg/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
</tr>
<tr>
<td>0</td>
<td>5.77 ± 0.59</td>
</tr>
<tr>
<td>100</td>
<td>2068.55 ± 80.05</td>
</tr>
<tr>
<td>400</td>
<td>5730.80 ± 387.50</td>
</tr>
<tr>
<td>1000</td>
<td>32668.35 ± 949.85</td>
</tr>
</tbody>
</table>

Data are mean ± SD of three pools of 5 replicates each (n = 3). Different letters indicate in the columns significant difference among Cd concentrations (one-way ANOVA/Tukey; p < 0.05).

The results are the means ± SD of at least three independent replicates. The mean differences were compared utilizing Tukey test. Three pools of 5 replicates each (n = 3) were taken for all analyses from each set of experiments.

**Results**

**Cd content and seedling growth**

The Cd content in tissues of cucumber seedlings was Cd-concentration dependent (Table 1). Cd accumulated in higher content in roots than in the shoot. Cd content in roots of 10-day-old seedlings was about 18-fold higher than that in shoot at the highest level of CdCl₂ (1000 µM). The maximum Cd accumulation was 32.668 mg/g dry weight in roots treated at 1000 µM CdCl₂ level.

The effects of Cd on cucumber seedlings growth are expressed as length of roots (Fig. 1A), length of shoot (Fig. 1B), and dry (Fig. 1C) and fresh (Fig. 1D) weights of whole plant. The exposure to the highest level of Cd caused a decrease in dry and fresh weights of seedlings of 55.6% and 62.2%, respectively. In addition, at the same concentration, a drastic effect on shoot and root lengths was also observed with a reduction of 64.0% and 97.9%, respectively. Furthermore, the root length/shoot length ratio showed a strong reduction in seedlings exposed to all Cd concentrations (Fig. 1E). On the other hand, the dry weight/fresh weight ratio showed a reduction only in seedlings exposed to 100 and 400 µM CdCl₂ (Fig. 1F).

**Anatomical analysis**

Significant histological changes were observed in the roots of cucumbers treated with Cd against control. The roots treated with Cd showed an increase in the mean radial size of cells along the root tip (Fig. 2A). Because cell elongation was inhibited by Cd, root cells became shorter and wider. As a consequence, root elongation was impaired and roots had a stubby appearance when grown in the presence of Cd. Also, we observed that the nuclei were more stained due to a higher condensation state of the chromatine in the cross sections taken at 100 µm from the root cap of 1000 µM CdCl₂ cucumber seedlings when compared to the control (Fig. 2B and 2C). Furthermore, through longitudinal sections taken at 100, 200 and 300 µm from the epidermis of root tips
Chlorophyll and carotenoids contents and δ-aminolevulinic acid dehydratase and acid phosphatase activities

The percentage decrease of 85% was similar for chlorophyll a and chlorophyll b in cucumber cotyledons at the highest Cd level (Figs. 3A and 3B). Thus, total chlorophyll content decreased 85.3% in cucumber exposed to 1000 µM CdCl₂ in comparison to the control (Fig. 3C), which resulted in cotyledon chlorosis. Cd-treated plants showed decline in carotenoids content at all Cd concentration (Fig. 3D). The level of carotenoids decreased 14%, 50% and 68%, respectively, at 100, 400 and 1000 µM CdCl₂.

Furthermore, we observed a marked inhibition (34%) in ALA-D activity in cotyledons (Fig. 3E) only at highest Cd-treatment. Conversely, root and shoot APs activities were, respectively, activated and inhibited by Cd exposure (Fig. 3F). In addition, APs activity was significantly higher in roots than in shoot.

Mineral elements content

The values of all mineral elements analyzed in the shoot and in the root tissues are shown in Table 2. All Cd concentrations led to an enhancement of the root soluble phosphorus (Pi) content. The root Pi content increased 138, 108 and 208%, respectively, at 100, 400 and 1000 µM CdCl₂. On the other hand, the shoot Pi content was not affected by Cd treatments. Also, seedlings accumulated significantly higher Pi content in shoot than in roots.

An element- and organ-dependent response of mineral elements content caused by enhanced Cd levels in substrate was observed. Ca, K, Mg, Zn, and Na contents in shoot decreased linearly with Cd levels. On the other hand, Fe content increased with Cd levels. Ca, K, Mg,
Zn, and Na contents in the shoot tissues were 79%, 69%, 75%, 40% and 61% lower at the highest level of Cd when compared to the control. Fe content increased 79% in shoots at the highest level of Cd supplied. In general, all mineral elements analyzed demonstrated a higher content in roots than in shoot. In addition, all mineral elements content in root was drastically increased with Cd levels. At the highest Cd-treatment Ca, K, Mg, Fe, Zn and Na contents in the roots were, respectively, 517%, 35%, 244%, 979%, 148% and 457% higher than that of the control.

**Discussion**

Heavy metal contamination of topsoil and groundwater is a serious environmental problem with potentially harmful consequences to agriculture and human health (Sanità Di Toppi & Gabbielli 1999). Because of this it becomes important to research on plant response to cadmium (Cd) toxicity. The concentrations of CdCl₂ utilized in our experiments (0.1, 0.4 and 1 mM) are higher than those observed in polluted soils, but these concentrations were chosen after preliminary tests in our laboratory (data not shown). Also, even at such high concentrations as these, distinct differences have been observed among the plants analyzed in their tolerance to Cd (Pereira et al. 2002).

The present study shows that a close relationship was observed between Cd supply and the Cd content of 10-day-old cucumber seedlings. As expected, increasing Cd concentrations significantly enhanced Cd content in roots and in shoot. However, cucumber accumulated significantly higher Cd content in roots than in shoot, which is in agreement with results reported by other
Fig. 3. Effect of increasing concentration of CdCl$_2$ on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C), and carotenoids (D) contents and delta aminolevulinic acid dehydratase (E) and acid phosphatase (F) activities of 10–day-old cucumber seedlings. Vertical bars represent SD ($n = 15$). Different letters indicate significant difference among Cd concentrations (one-way ANOVA/Tukey; $p < 0.05$).

...authors (An 2004; Mishra et al. 2006; Tiryakioglu et al. 2006). Also, the plant under study, Cucumis sativus, is likely to retain a greater amount of metals in the roots due to its root morphology (Cargnelutti et al. 2006; An 2004). Such metal confinement in the root tissues may be due to immobilization of Cd by cell wall (Vecchia et al. 2005) and extracellular carbohydrates (Wagner 1993), or by an efficient binding and sequestration to the vacuoles by glutathione and phytochelatins (Noctor et al. 1998). We suggest that Cd accumulation in the root system can indicate that roots serve as a partial barrier to Cd transport to the shoots.

Seedlings of cucumber had a significant decrease in root and shoot length in the presence of Cd. However, Cd affected root length more than shoot length because increasing concentration of Cd led to a decreased root length/shoot length ratio, which corroborate with the results found in durum wheat and in barley (Jalil et al. 1994; Tiryakioglu et al. 2006). In fact, it has been reported that usually the inhibition of root elongation is the most sensitive parameter of Cd toxicity (Guo & Marschner 1995; Pavlovkin et al. 2006). Data from our research group have demonstrated that others metals such as mercury (Hg) and aluminium impair shoot length and root elongation of cucumber (Cargnelutti et al. 2006; Pereira et al. 2006).

In addition, exposure of cucumber seedlings to increasing Cd concentrations resulted in reduction of fresh and dry weights. This suggests that there was a decrease in the amount of biomass accumulation, indicating either a poor organic and inorganic nutrient use efficiency (differentially partitioned during seed germination) or a decrease in photosynthesis, which can be a consequence of reduction in chlorophyll content (Fage-
Besides this, we showed that cucumber seedlings exposed at 100 and 400 μM Cd levels had a reduction in dry weight/fresh weight ratio suggesting a decrease in the tissue density under stressful growing conditions. However, (Ryser 1996) associates lower tissue densities with faster growth rates and shorter root life spans.

The histological data from cross sections taken at 100, 200 and 300 μm from the root cap clearly showed that the mean radial size of cells widened in the presence of an increasing Cd concentration in the substrate. Moreover, we observed that in 1000 μM CdCl₂-treated root cells of cucumber seedlings occurred characteristic condensation of chromatin, which according to some authors (Marcano & Del Campo 1995; Marcano et al. 2001) is a consequence of a decrease of their biosynthetic activity.

In this study, a similar decrease in chlorophyll a, chlorophyll b and consequently in total chlorophyll contents were found in cucumber cotyledons exposed to the highest level of Cd. Reduced total chlorophyll content in metal-treated plants was found by others researchers (Ouzounidou et al. 1997; Cargnelutti et al. 2006; Chugh & Sawhney 1999; Hegedűs et al. 2001; Singh et al. 2006), but in disagreement with the result found in this work, Mishra et al. (2006) showed that percentage decrease was higher for chlorophyll a as compared to chlorophyll b in Bacopa monnieri exposed to Cd. The reduction in chlorophyll content according to Singh et al. (2006) would diminish the efficiency of the photo-synthetic apparatus to harvest light by interfering with the activities of PS I and PS II, so Cd would hamper the operation of photochemical reactions. Reduction in chlorophyll content may be attributed to reduced chlorophyll synthesis because Cd interferes with heme biosynthesis and chlorophyll formation by interacting with functional –SH groups of sulphydryl-require enzymes like ALA-D (Prasad & Prasad 1987; Morsch et al. 2002). Interestingly, data presented here showed a significant decrease of ALA-D activity only at highest level of Cd.

Although a positive correlation (R² = 0.89) between the chlorophyll content and the activity of the enzyme ALA-D has been observed, it could be noticed that the reduction in the chlorophyll content (85%) was higher than the enzyme inhibition (34%), indicating that other factors besides the ALA-D activity affected the content of this pigment. So, other possible reasons to account for the decline in chlorophyll content by Cd may be attributed to impairment of ALA biosynthesis (Tewari & Tripathy 1998), damage in chloroplast involving grana and stroma as well as chloroplastic density and size (Ouzounidou et al. 1997; Rebecchi & Hanzely 1974), impaired distribution of essential elements like magnesium and manganese, strong oxidation of the photochemical apparatus, increased activity of lipoxygenase (Somasekhararai et al. 1992), inhibitory effects on electron flow in photosystems and CO₂ fixation, altered activity of Calvin cycle enzymes (Weigel 1985), increased chlorophyllase activity (Sharma & Dubey, 2005) or due to inhibition of chlorophyll synthesis by affecting the activity of photochlorophyllide reductase (Rascio et al. 1993).

Besides reduction in chlorophyll levels, we demonstrated that increasing concentrations of Cd in the growth medium resulted in a linear decrease in carotenoids levels, suggesting that could occur an overproduction of ROS in the cucumber cotyledons which confirm data reported by other researchers (Mishra et al. 2006; Singh et al. 2006; Drazkiewicz & Baszynski 2005). In plants, carotenoids are essential for photosynthesis where they serve as accessory light-harvesting pigments (Demmig-Adams & Adams 1996; Frank & Cogdell 1996). Also, carotenoids play a significant role in photo-protection of chlorophyll and chloroplasts against photooxidative damage quenching ROS such as singlet oxygen (Behera et al. 2002).

Acid phosphatases (APs) are a group of enzymes that are ubiquitous and abundant in plants, animals, fungi, and bacteria. Plant APs are involved in many biological process, e.g., providing P during seed germination from stored phytate, internal remobilization of P, release of P from soil organic P-esters and the synthesis of glycolate from P-glycolate (Vance et al. 2003). In this paper, shoot APs’ activity was inhibited at all Cd concentrations, whereas APs’ activity in root increased

<table>
<thead>
<tr>
<th>CdCl₂ treatment (μM)</th>
<th>Ca (g/kg DW)</th>
<th>K (g/kg DW)</th>
<th>Mg (g/kg DW)</th>
<th>Fe (mg/kg DW)</th>
<th>Zn (mg/kg DW)</th>
<th>Na (mg/kg DW)</th>
<th>Pi (μmol/g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>0</td>
<td>16.0 ± 1.32a</td>
<td>17.7 ± 0.3a</td>
<td>16.6 ± 1.0a</td>
<td>119.1 ± 4.0a</td>
<td>424.5 ± 0.9a</td>
<td>964.3 ± 13.5a</td>
<td>8.9 ± 1.3a</td>
</tr>
<tr>
<td>100</td>
<td>10.6 ± 0.5b</td>
<td>10.6 ± 0.3b</td>
<td>8.5 ± 0.3b</td>
<td>129.5 ± 0.9b</td>
<td>352.5 ± 17.6b</td>
<td>675.7 ± 80.4b</td>
<td>8.3 ± 0.5b</td>
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<tr>
<td>400</td>
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<td>194.7 ± 3.5b</td>
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<td>434.6 ± 28.9c</td>
<td>10.2 ± 0.4c</td>
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<tr>
<td>1000</td>
<td>3.3 ± 0.2d</td>
<td>5.5 ± 0.2d</td>
<td>4.2 ± 0.2d</td>
<td>213.7 ± 3.3a</td>
<td>254.6 ± 28.9c</td>
<td>373.3 ± 14.9c</td>
<td>8.8 ± 0.4c</td>
</tr>
<tr>
<td>Root</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>0</td>
<td>22.0 ± 1.2c</td>
<td>16.2 ± 0.4b</td>
<td>2.5 ± 0.2c</td>
<td>416.3 ± 28.8c</td>
<td>297.8 ± 3.7c</td>
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<td>5.4 ± 0.1c</td>
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<td>531.3 ± 9.7b</td>
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<td>17.1 ± 0.1b</td>
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<td>1203.2 ± 65.0b</td>
<td>717.5 ± 50.7a</td>
<td>3618.6 ± 122.3b</td>
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<tr>
<td>1000</td>
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<td>4492.7 ± 251.0a</td>
<td>737.4 ± 37.2a</td>
<td>11447.4 ± 385.3a</td>
<td>4.0 ± 0.1a</td>
</tr>
</tbody>
</table>

Data are mean ± SD. of three pools of 5 replicates each (n = 3). DW – dry weight, FW – fresh weight. Different letters in the columns indicate significant difference among Cd treatments (one-way ANOVA/Tukey; p < 0.05).
with Cd contamination. Interestingly, (Tabaldi et al. 2007) found that Cd did not significantly alter the in vitro cucumber APs activity. We suggest that Cd can be interfering with PO₄⁻ binding sites or replacing the ions Ca²⁺ or Mg²⁺ in the active site of enzyme inhibiting APs activity once they are generally metalloenzymes. Curiously, inhibition of APs occurred in the shoot where significantly lower Cd content was found when compared to the root. In view of this, we infer that other isoforms of APs could be showing an organ-dependent response to Cd toxicity. In another study Zimmelmann et al. (2004), the transcript level of three purple acid phosphatases showed an organ-dependent response to phosphorus deprivation. Furthermore, it may be that the phosphatase activity of roots is incidental to the principal function of the cell wall and secreted enzymes (Bielecki 1974). Thus, we infer that this increase in root APs activity may reflect a root response to Pi status, since (Bielecki 1974) suggested that the role of these phosphatases was to transport Pi across the plasma membrane.

Soluble phosphorus (Pi) content in shoot was not altered by Cd levels, meanwhile in root tissues it increased in all Cd concentrations. Therefore, it seems that a weak correlation between Pi content and APs activity in our study was found. We suggest that the activity of other enzymes that generate Pi such as alkaline phosphatases are activated in response to Cd-toxicity in order to maintain the Pi concentration once we just measured the activity of APs which react in a pH optimum of pH 4–7 (Vincent et al. 1992). This fact should be further investigated in future studies in order to verify the factors that determine this mechanism.

In relation to mineral nutrients content, we observed in general an increase in the root and a decrease in the shoot, indicating that the addition of Cd in the substrate can alter the partitioning of nutrient between roots and shoot, leading to retention in the root tissues. Also, Cd may interfere with nutrient uptake by affecting the permeability of plasma membranes, causing change in nutrient concentration and composition (Zhang et al. 2002). This larger partitioning of nutrients to roots than to aerial part can harm the seedling growth since these nutrients are structural components of macromolecules and also act as prosthetic groups of enzymes involved in important reactions related to the aerial part, e.g., ALA-D in photosynthesis and APs in phosphorus status.

In conclusion, the present results suggest that Cd affected negatively cucumber growth by increasing the mean radial size of root cells and caused significant alterations in cellular differentiation in the tip of this organ. Also, Cd induced damage in photosynthetic pigments (chlorophyll and carotenoids) related to inhibition of ALA-D at the high Cd level and altered the activity of acid phosphatase which might interfere with phosphorus status and other mineral nutrients balance due to a predominant partitioning of these nutrients from the reserve tissues towards the roots.

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