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The effect of cadmium (Cd) on growth, and photosynthesis of tomato at seedling stage

Patrick Okori, Cosmos Magorokosho and Thokozile Ndlela

Research Unit, Nutrition and nitrogen metabolism and stress protein (99UR/09-20),Department of Biology, Faculty of Sciences of Tunis, Campus Universitaire El Manar I,1060, Tunis-Tunisia.

Abstract

The effect of cadmium (Cd) accumulation on mineral composition in roots and shoots, growth development, chlorophyll and water content of tomato (*Solanum lycopersicon* Mill 63/5F1) seedlings was tested under doses of 0 (control), 10, 50 and 100 µM. Cadmium accumulation was defined by elevated concentrations as 50 to 100 µM in root and shoot tissues. Element uptake by roots and shoot was negatively affected by raise in cadmium concentrations, especially in 100 µM Cd. Root and shoot elongation, root and shoot fresh and dry biomass, leaf area were negatively affected by increasing Cd concentrations. Tissue water content, growth tolerance index, chlorophyll a, chlorophyll b and total chlorophyll content were limited by cadmium toxicity. The addition of Cd in the growth medium also had significant deleterious effect on net photosynthetic rate (*Pn*) and intracellular CO_2 concentration (*Ci*), with *Pn* being reduced by 27.2 and 62.1% at 5 µmol/L and 100 µM Cd treatments compared to the control, respectively, while *Ci* increased correspondingly by 28.4 and 39.3%.

Keywords: Cadmium (Cd), toxicity, growth, nutrient, chlorophyll, photosynthesis.

INTRODUCTION

Cadmium is a major pollutant in both terrestrial and aquatic several decades (Fishbein, 1981). This is critical in soils ecosystems. Besides natural weathering process the used for agricultural production, because pollutants can main sources of Cd pollution are exhaust fumes of be accumulated in crops. This can cause threats to automobiles, chimneys of factories using Cd, effluents human health (Sharma and Dubey, 2005), and exceed from the storage battery, industry, mining and smelting of legal limits. Lead naturally occurs in soils but is in relatively low pesticides and additives in pigments and gasoline concentrations. Lead concentrations in uncontaminated (Papadopoulos and Rowell, 1988). Cadmium is widely distributed in the earth's crust (0.1-0.5 µg. g^{-1}), the atmosphere (1 - 5 ng. m^{-3}), marine sediment (1 µg. g^{-1}) and sea water (0.1 µg. L^{-1}). Soil is regarded as it will exceed the legal limit if the pH is below 6.0 (Raskin significantly affected with a possibility that food grown on et al., 1997). Although cadmium is not an essential element for plants, it gets easily absorbed and accumulated in different plant parts. Excess Cd causes a number of toxicity symptoms in plants, sure as stunted growth, chlorosis, and blackening of root system. Vegetables growing in medium with high level of Cd showed deleterious effect in photosynthetic processes, such as chlorophyll content and photosynthesis (Fatoba and Udoh, 2008), upsets mineral nutrition and water balance, changes hormonal status and affects membrane structure and permeability (Sengar et al., 2008). However, results of these investigations are rather contradictory, as the nature of heavy metal effect depends on the species, variety and age of plants and the concentrations, duration of effect, physical and chemical properties of contaminants. Therefore plant cultivation in contaminated soils is problematic due to hyper accumulation of HM in fruits and vegetables (Sahmurova et al., 2010). The effects of heavy metals must be investigated in all aspects of every cultivated

species.

The aim of this study is to define cadmium effects on the accumulation and distribution of cadmium and other elements in roots and shoots, and growth and development of plant organs, water contents of tissues and chlorophyll amounts of leafs in tomato. The present study was undertaken to investigate the effect of Cd on growth, and photosynthesis of tomato at seedling stage, in order to clarify physiological effect of Cd stress on photosynthesis in tomato plants.

MATERIALS AND METHODS

Plant material

Seeds of tomato (*Solanum lycopersicon*. Mill cv 63/5F1) were sterilized in 10% (v/v) hydrogen peroxide for 20 min, and washed abundantly in distilled water afterwards. After imbibition, the seeds were germinated on moistened filter paper at 25°C in the dark. After 7 days, uniform seedlings were transferred to 6 litres plastic beakers (8 plants per beaker) filled with continuously aerated, basal nutrient solutions of an initial pH 5.8-6, containing 3 mM KNO_3, 0.5 mM Ca $(NO_3)_2$, 2.4 mM KH_2PO_4, 0.5 mM $MgSO_4$, 100 µM Fe-K_2-EDTA, 30 µM H_3BO_3, 5 µM $MnSO_4$, 1 µM $CuSO_4$, 1 µM $ZnSO_4$, and 1 µM $(NH_4)_6$ Mo_7O_{24}. Plants were grown in a growth chamber (26°C/70% relative humidity during the day, 20°C/90 % relative humidity during the night). The photoperiod was 16 h daily with a light irradiance of 150 µmol $m^{-2}.s^{-1}$ at the canopy level. At the age of 10 days after transplant, cadmium was added to the medium as $CdCl_2$ at 0, 5, 10, 50 to 100 µM. After one week of Cd treatment, plants were separated into shoots and roots. Samples were stored in liquid nitrogen for subsequent analysis or dried a 70°C for at least three days in order to determine both dry material and ionic contents.

Plant growth, biomass and growth tolerance index

Seedlings were harvested, washed with dionised water and separated into roots and shoots at the end of the study. Washed seedlings were % valuated for elongation, fresh and dry biomass and area of shoots. Dry biomass was determined after oven drying the samples at 65°C for 2-3 days until a constant was weight gained. Cd tolerance was calculated as the growth tolerance index (GTI) which gives the integrated percentage of Cd-treated to Cd-untreated control seedling parts, and it gives an opinion and or effect of applied stress factor on plant grown and development (Rini et al., 2008).

Measurement of mineral elements

Five plants were taken from each treatment and separated into roots and shoots. The shoots and roots were washed in tap and deionized water three times and dried at 65°C for 48 h. The dried tissues were weighed and ground into a fine powder before wet digestion in $HClO_4$:HNO_3 (4:1, v:v) solution. Calcium, magnesium and potassium were determined by atomic absorption spectrophotometer (Perkin Elmer 3100, USA) of plant part (Likens et al., 1967).

Cadmium accumulation

Total shoot and root accumulation of Cd in *Solanum lycopersicon* were determined after 10 days of treatment. Roots and shoots were harvested, washed in deionized water for 2 min, air dried for 2 days, and then ground into a fine powder using a pestle and mortar. A known amount of this powder was dissolved in 3 parts of 1 M HNO_3 and 1 part of 1 M $HClO_4$, and the metal concentration in solution was analyzed by atomic absorption spectrophotometry (Philips PYE Unicam PU 9000).

Chlorophyll contents

Shoot collected at day 17 after Cd treatment were weighed and ground in 80% acetone. The resulting suspension was centrifuged for 10 min at 5000 rpm. The chlorophyll contents of supernatant were estimated according to Arnon (1949). Tissue water content (TWC) was obtained from the fresh weight-dry weight/ dry weight ratio (Raja et al., 2006).

Photosynthetic parameters

Net photosynthetic rate (*Pn*), stomatal conductance (*Gs*) and intracellular CO_2 concentration (*Ci*), were determined using an LCi (leaf chamber analysis) portable photosynthesis system (ADC, Analytical Development Company, England). All measurements were performed on the upper second fully expanded leaves.

Statistical analysis

The results are the means±S.E. of at least three independent replicates. The analyses of variance were computed on statistically significant differences determined based on the appropriate *F*-tests. The mean differences were compared utilizing Duncan's multiple range test.

RESULTS

Cadmium uptake and accumulation

All lead applications caused an increase in Cd concentrations root and shoot tissues (Figure 1). Low cadmium concentration increased shoot cadmium contents of the tissues of tomato seedlings (Figure 1A). Exposure to excess cadmium, particularly moderate, and high Cd, led to more increased accumulation of Cd in roots and shoots. Cadmium levels of plant were found to be 10 in roots and 3.57 µmol/g DW in shoots at low Cd concentration (Figure 1A and B). In addition, the cadmium levels of plant were found to be 51.127 in roots and 12.34 µmol/g DW, in shoots at the high Cd applications.

Elements uptake

Analysis of variance indicated that macro elements accumulations in roots and shoots of tomato seedlings were affected by excess Cd applications (Figure 2). The highest level of cadmium generally inhibited the uptake of all mineral elements compared with the low cadmium treatment. Calcium and magnesium concentrations of

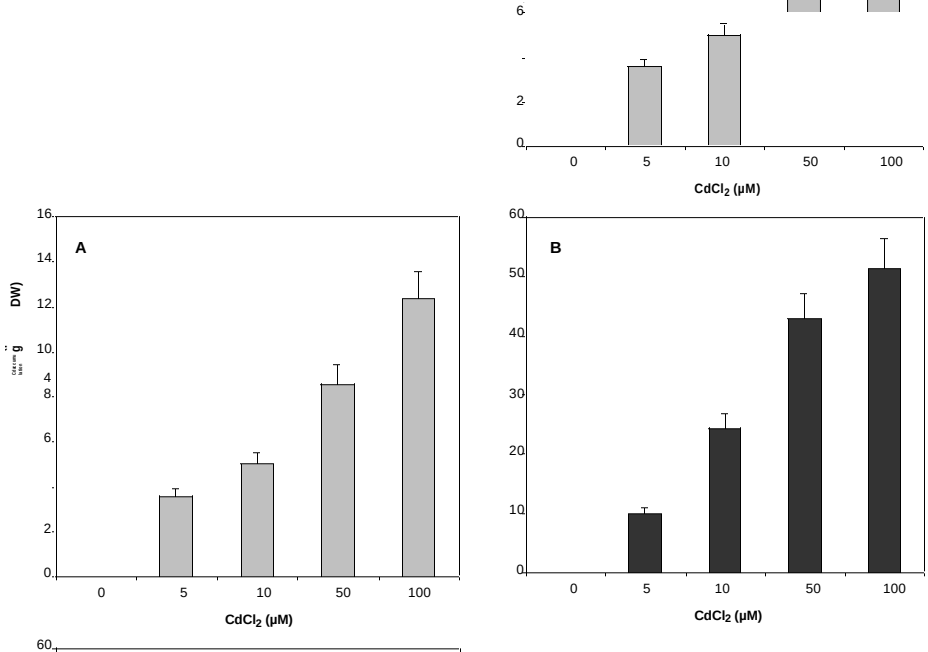

Figure 1. The effect of different cadmium concentrations in the cadmium accumulation on shoots (A) and roots (B) of tomato plants. Values are the means ± SE of triplicates from five independent experiments.

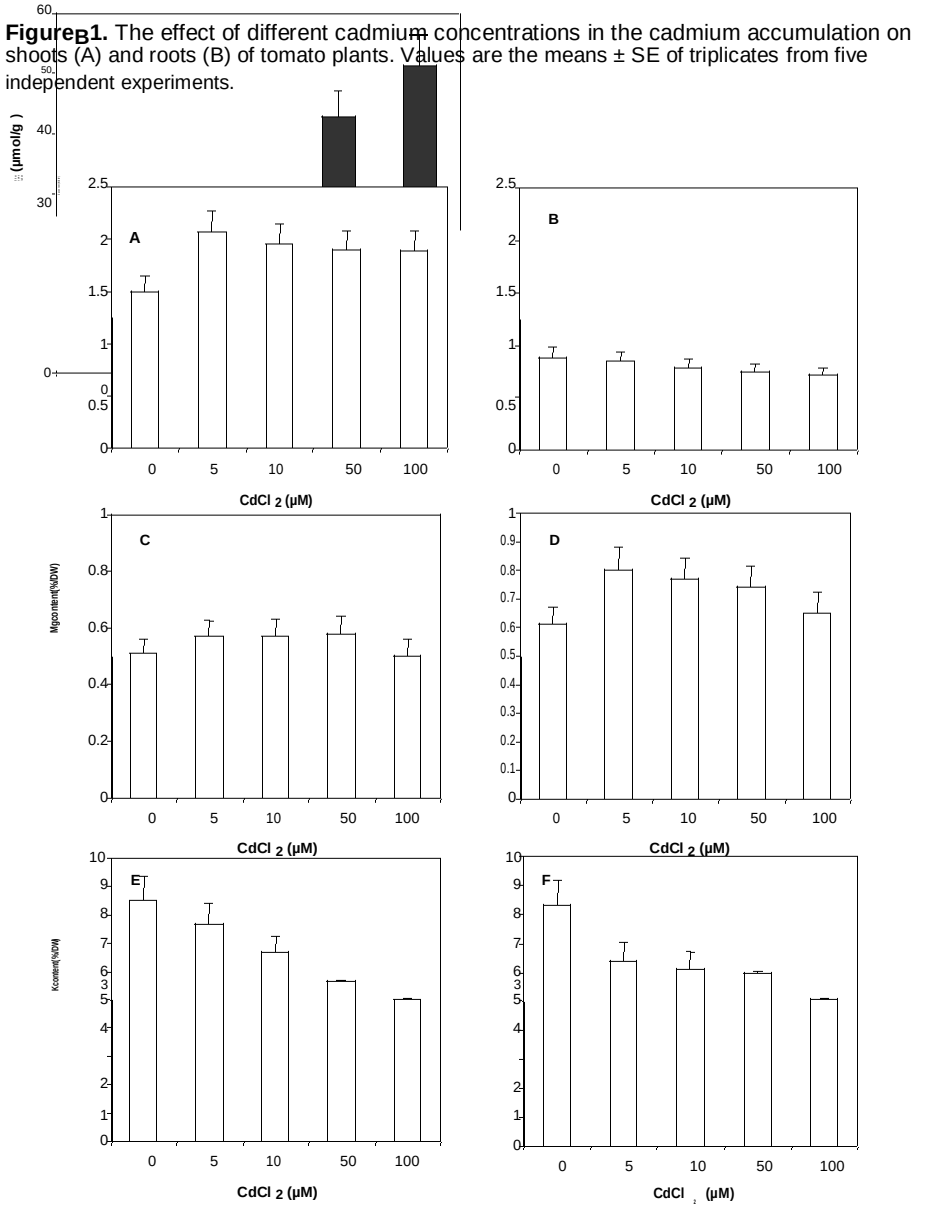

Figure 2. The effect of different cadmium concentrations on shoots (A) and roots (B) calcium content, on shoots (C) and roots (D) magnesium content and shoots (E) and roots (F) potassium content. Results were expressed on %DW. Values are the means ± SE of triplicates from five independent experiments.

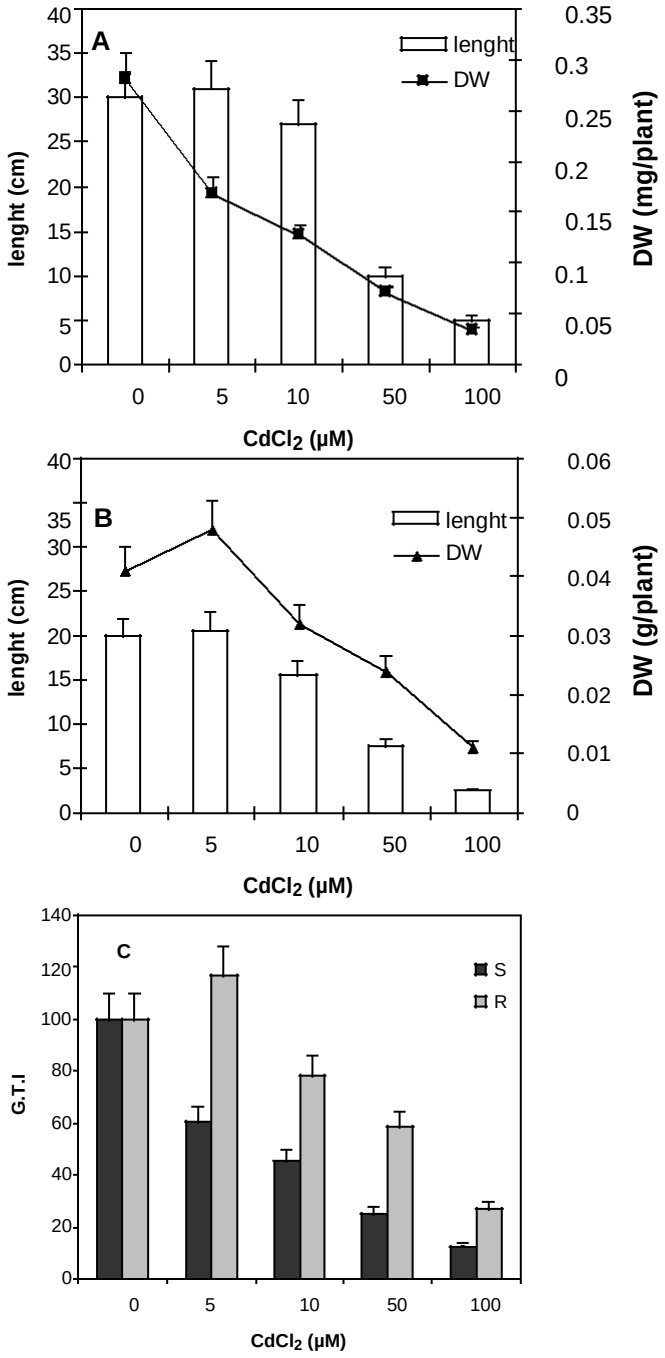

Figure 3. Effect of different cadmium concentrations on shoots (A) length and dry weight, on roots (B) length and dry weight and on shoot and root growth tolerance index (C) Values are the means ± SE of triplicates from five independent experiments.

shoots was significantly decreased with increasing Cd concentration (Figure 2A and C). Calcium and magnesium content of roots were not significantly different at all cadmium levels (Figure 2B and D). in the potassium content in roots and shoots level, cadmium treatment decreased with only 100 µM Cd level

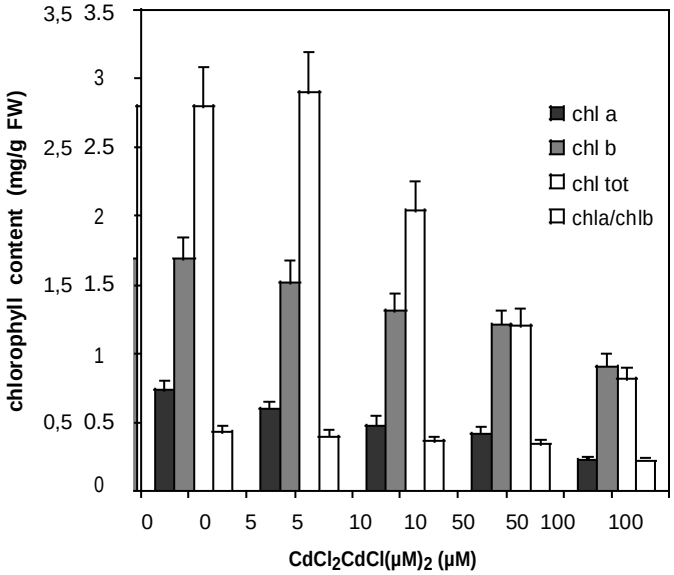

Figure 4. The effect of different cadmium concentrations on shoots chlorophyll *a*, chlorophyll *b*, chlorophyll *total* content and report chl *a*/chl *b*. Values are the means ± SE of triplicates from five independent experiments.

compared with control (Figure 2E and F). A difference was not obtained between control with low and moderate Cd levels.

Plant growth, biomass and growth tolerance index

Excess Cd concentrations restrained growth parameters (Figure 3). Length and dry weight of root and shoot were decreased compared to the controls (Figure 3A and B). These results showed that cadmium contamination negatively affected root and shoot tissue. Growth tolerance index (GTI) is an integrated calculation of particular parameters and makes for a summary assessment of effect of stress factor on plant growth and development (Figure 3C). GTI was affected by cadmium, that is the applied stress factor in our study, $P<0.001$. The GTI of plants was calculated based on morphological parameters and showed inhibition of growth and biomass synthesis in all lead concentrations. The value of GTI was 100 in controls while 12.8 in shoots and 26. 82% in roots at 100 µM Cd.

Chlorophyll, water content and photosynthesis rate:

Chlorophyll *a*, *b*, *tot*, were affected negatively by increasing Cd levels. Chlorophyll *a* ($P<0.001$), chlorophyll *b* ($P<0.05$) and chlorophyll *tot* ($P<0.001$) was significantly affected by increasing cadmium concentrations (Figure 4). Root and shoot water contents were decreased in all Cd levels compared with control (Figure 5). Loses of the

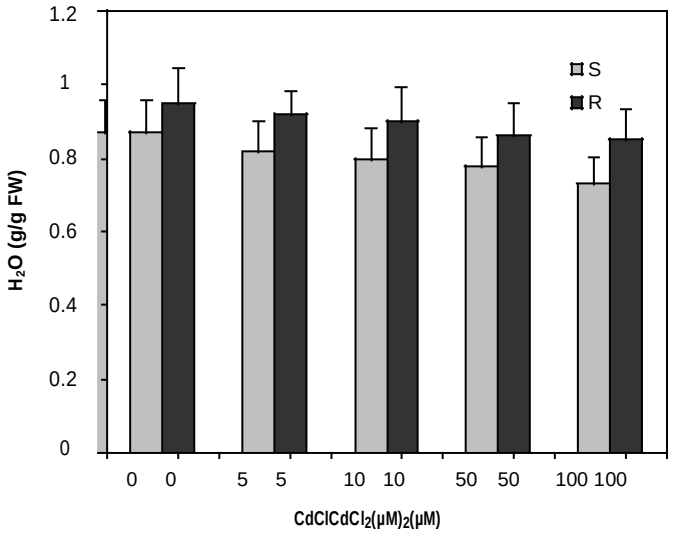

Figure 5. The effect of different cadmium concentrations on shoots and roots water content. Values are the means ± SE of triplicates from five independent experiments.

water content in root were by 10.52% at 100 µm Cd according to control. Loses in shoots were more than in roots as 16.09% at low at high Cd levels.

Photosynthesis parameters

As shown in Figure 6, after Cd exposure, *Pn* decreased nearly linearly with increasing Cd in nutrient solution for tomato plants. In plants treated with 100 µM Cd, *Pn* was lowered by 86.982%, compared with controls (Figure 6A). As to stomatal conductance (*Gs*), plants treated with 5-10 µM Cd showed significant decrease, while a slight increase was observed in the plants exposed to 50 and 100 µM Cd (Figure 6B). In contrast, the intracellular CO_2 concentration (*Ci*) increased with increasing Cd concentrations in nutrient solution of tomato plants (Figure 6C).

DISCUSSION

Excess cadmium caused accumulation of Cd in tissues of tomato seedlings. While the Cd concentration increased, the distribution of Cd within the plant followed the trend root>shoot. At the high cadmium concentrations, roots accumulated 4.16 fold more cadmium than shoots. There must be different mechanisms for uptake and accumulation for cadmium in general and for the different plant parts. So, root tissue acts as a barrier to Cd translocation to the shoot. Our results were partly compatible with other findings that Cd was stored in tomato roots. This event can be useful for the environment. Actually high cadmium deposition in corn

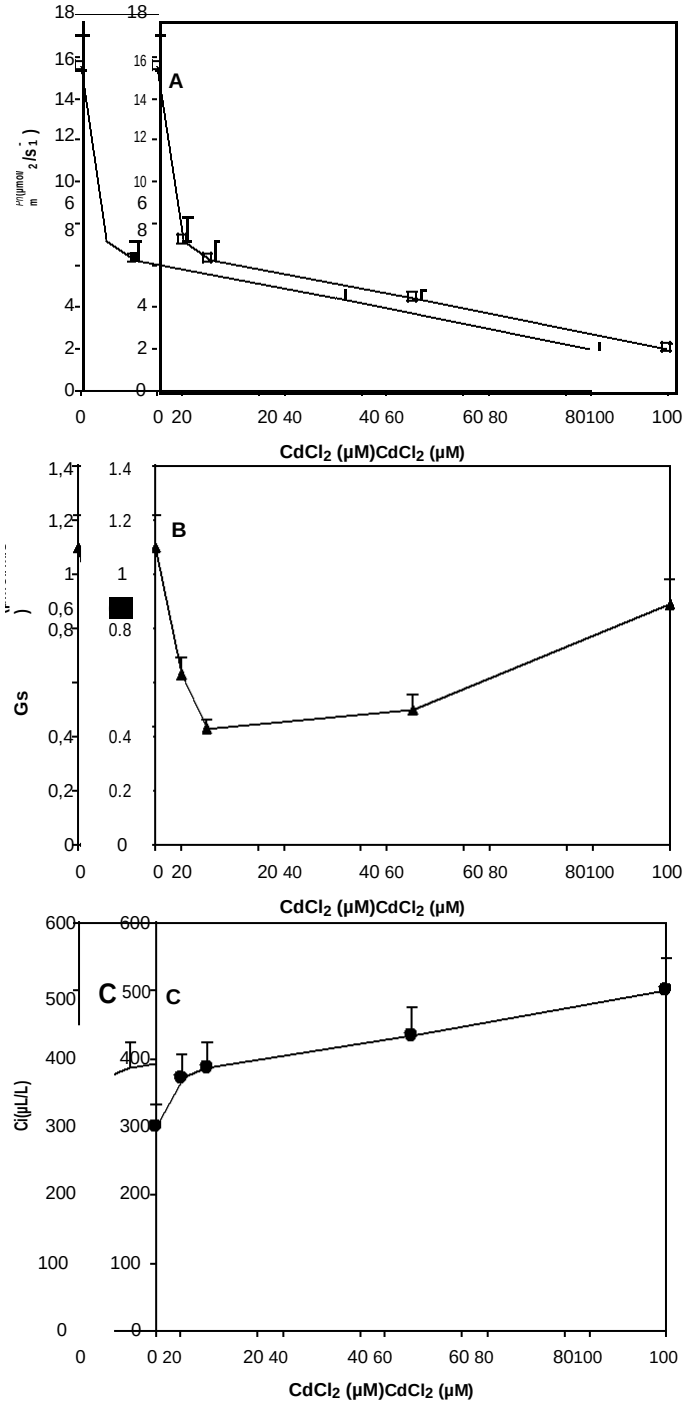

Figure 6. The effect of different cadmium concentrations on net photosynthetic rate (A), on stomatal conductance (B) and on intracellular CO_2 concentration (C). Values are the means ± SE of triplicates from five independent experiments.

root tips was observed than in upper organs (Malkowski et al., 2008). So, they pointed that rhizofiltration is the use of plant roots to remove contaminants, as heavy metals from contaminated water can have implications for this emerging environmental cleanup technology.

There is little information about Cd stressed tomato on mineral accumulation in plants. The metal concentration in roots and shoots of the tomato under study may be influenced by an exclusion mechanism triggered by high external Cd concentrations, resulting in a reduction in root and shoot metal concentrations. The levels of calcium showed significant decreases with Cd applications, especially at 100 µM Cd. The levels of magnesium showed significant decreases in shoots of tomato. The levels of potassium in roots and shoots showed significant decreases with increasing Cd applications. There must be different mechanisms for uptake for macro elements for the different plant parts under Cd press. Another work showed that Ca and Mg inhibited the transport of Cd in rice roots (Kim et al., 2002). These results are similar to our results with Ca and Mg contents decreasing in root at moderate and high Cd applications. The growth, dry weight and growth tolerance index of root and shoot were negatively affected by increasing cadmium concentrations in tomato seedlings. Similar results were obtained by some other studies at the evaluated Cd concentration, root and shoot growth and dry biomass is significantly reduced in *Pisum sativum* (Becerril et al., 2001), and in *Lycopersicon esculentum* (Chaffei et al., 2003).

Tomato seedling treated with Cd resulted in a decrease in growth tolerance index (GTI). Tolerance index can be accepted strength ability to stress factor that generally indicates growth in plants in plants metal treated as a percentage of the control in most stress investigations. Tolerance index is also reduced with pressure of increasing Cd in medium culture (Metwally et al., 2005). Cadmium disrupts photosynthesis in different ways. The decline of the photosynthetic rates results from the distorted chloroplast ultra structure, the restrained synthesis of chlorophyll, the obstructed electron transport, the inhibited enzyme activities of the Calvin cycle and CO_2 deficiency was due to stomatal closure. All the metabolic changes produced by Cd listed above dramatically modify plant growth and development.

Morphogenetic distortions are a non-specific symptom of the effects exerted by diverse stress factors convenient for assessing the phytotoxicity of these factors (Dong et al., 2005).

The results of this paper shows that phytotoxicity of increasing Cd 5 to 100 µM, which is apparent from the reduction of cholorophyll concentration in plant. One the direct effects includes the inhibition of chlorophyll. The inhibition of chlorophyll synthesis by heavy metals is often manifesting as chlorosis. All these changes produced by Cd dramatically modify plant growth and development. The spectral change due to enhancement of Cd dose, led to significant reduction in chlorophyll biosynthesis. This may be related with accumulation of Cd in tissues. Negative effect was determined in the Cd concentration on tissues water contents in this study.

On the other hand, intracellular CO_2 (*Ci*) in tomato seedlings was significantly increased with increasing Cd level in the nutrient solution. Ainsworth and Rogers (2000) reported that, reduction of CO_2-exchange rate could not be completely explained by any single factor and appeared to be due to the integrated effect on stomatal conductance, chlorophyll content and on function of photosynthetic apparatus. In addition, the reduced CO_2 fixation in Cd-treated wheat seedlings was not accompanied by decreased stomatal conductance[18]. In contrast, Vassilev et al. (2002) reported that the disturbed water relations to plants comprised one of the main reasons for the heavy metal phytotoxicity. So it may be assumed that the decrease in Pn is ascribable to the inhibition of various reaction steps in the Calvin cycle, the Hill reaction and CO_2 fixation (Dong et al., 2005). However, further experiment should be done in order to have a better understanding of the mechanism of the effect of Cd toxicity on photosynthesis.

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