

Cadmium Toxicity: The Investigation of Cd Toxic Level in Different Organs of Cherry Tomato Plant and the Effect of Cd Accumulation

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This research was done in hydroponic environment in greenhouse at 3 stages (vegetative, flowering and full product) in 5 concentrations of Cd (0.1, 0.3, 0.9, 2.7, and 10 μm) for investigating physiological and biological effects. This study revealed that the increase of Cd concentration in understudy treatments causes the 49% reduction of sugar solution in 10 μm treatment. On the other hand, the gradual increase of Cd in cultivation caused the increase of starch, reduction of photosynthesis and blockage of carbon cycle enzymes. Furthermore, in heavy metal stress conditions of vegetative stage, Cd has negative effects on the protein amount of total treatments in $p=0.01$ signified level. When treatment starts, the amount of protein reduced until 33% in 0.1 μm concentration and this reduction can be seen also in other treatments. In contrast, in flowering stage the amount of protein is increased compared to the control. The study of different heavy metal concentration effects showed that plants are more sensitive to Cd (2-20 times more than others). The Generalized Linear Model variance test of aerial and underground plant organs traced the high level of Cd concentration in root in 10 μm concentration. This increasing process in $p=0.001$ level also was seen in root, leaf, and fruit. The accumulation of Cd expressed high speed of Cd transport from the soil to upper organs of tomato, and this process reduced the amount of solvable sugar and protein.

Abstract

Keywords: Accumulation, Cadmium, Heavy metal, Protein, Pollution, Sugar, Starch.

INTRODUCTION

Many parts of the world are located in arid or semiarid regions and lack of water supplies forces many countries use their sewage for irrigating their fields. There are many toxic elements in sewage such as As, Cd, Cu, and Pb. These elements are accumulated in soil horizon and their accumulations have been increased 3 to 6 times. When these toxic elements are changed to organic solutions or accumulated in environment due to hydrological or biological process, their concentrations would be increased. The increase of toxic element concentration is very hazardous for environment and public health. These toxic elements enter into biological cycle and bond with soil sediments and they are never omitted. The first signs of heavy metal contamination in plant are visible less than 30 minutes. These signs include the reduction of Ca^{2+} absorption, blockage of Ca^{2+} channels into plasma membrane, reduction of K^{+} diffusion, callus accumulation, discharge of malic acid, increase of phytochelatins, duplication of heat shock genes, and biosynthesis of heat shock protein. Many researchers believe that the toxic level of Cd is related to its bond tendency with SH group in enzymes and protein structures. In fact, the optimum growth of tomato is occurred in pH 5.5- 6 and acidic soil causes Cd accumulation and transport to different plant organs. This accumulation and transport have destructive effects on physiological quality of plant product.

This study can be a useful and applied research for genetic adjustment and production of plant varieties with less ability of Cd absorption. This research is done in order to measurement the effect of Cd in Cherry tomato in hydroponic culture environment. This study is based on qualitative and quantitative aspects including study of solvable/starch, protein concentration and the accumulation of Cd in different plant organs such as root, stem, and leaf.

Cd transport is done basically in 2 path ways: short distance transport in the width of the root cortex and long distance transport for stem in xylem and phloem. The most important matter is the accumulation of Cd in different parts of plant and use of probable mechanism for reduction of toxic effects of Cd and its destructive consequences on qualitative and quantitative aspects of plant. Carbohydrates are crucial macro molecules which are used in plant. By increase of Cd treatment in *Phyllanthus amarus*, it can be seen that the level of solvable sugar and photosynthesis reduced. In contrast, the amount of starch is increased. The accumulation of starch in treated plants is because of blockage of using sugar and starch. This matter is also can be seen in *Zea mays* and *Hordeum*. The reduction of sugar level is indirectly related to photosynthesis process and stabilization of CO_2 . By entering Cd into plant tissues, it causes reduction of carbon cycle enzymes. Bivalent metal cations have important role in Robisco action and balance between CO_2 and O_2 bounded by protein. Cd blocks the action of some enzymes such as fructose-1,6- bis phosphates and fructose-6 phosphate (Malik *et al.*, 1992).

Cd has a direct effect on photosynthesis. It indirectly reduces the level of sugar and increases the amount of starch. The study of distribution of carbohydrates in different organs of rice under heavy metal treatment showed that the total amount of carbohydrates in stem was 4 times higher compared to the control. On the contrary, the amount of carbohydrate was reduced in root and leaves (Moya *et al.*, 1993). Zenk and Kneer (1992) argued that in plants under stress of heavy metals, the action of enzymes used for protein production increased. There is a direct relationship between the level of Cd accumulation and synthesis of protein. Many researchers believe that plant species and their varieties have different absorption, accumulation, and resistance to heavy metals. Among leaf plants, ornamental cabbage is the one with high capacity of Cd accumulation. The leaves of cherry tomato absorbed 70 times more Cd compared to carrot leaves (Rezai, 2004).

The analysis of different heavy metal concentration revealed that after As, Cd has higher destructive power (Jana *et al.*, 1987). In plants under high Cd concentration treatment, the accumulation of this element was seen in roots, lower leaves, stems, and also upper leaves (Marufi

et al., 2005). There is a correlation between available Cd and its concentration in different plant organs. Cultivated plants in high Cd have more Cd in their root, stem, and leaves (Rezai, 2004). Excretion of Cd is equal to 29019×10 ton in a year. Using unpurified phosphate fertilizer is an important factor in increasing Cd concentration in soil. The standard amount of Cd in phosphate fertilizer is 15-25 mg kg⁻¹. On the other hand, the mean of Cd in soil is equal to 0.1 – 2 mg kg⁻¹ and its toxic level is 3 to 8 mg kg⁻¹. The normal amount of Cd in plants is 1 ppm and if it exceeds higher than 20 ppm, it causes the plant to be toxic. The standard amount of Cd (based on 2001/22/CE) in leaf vegetables such as ornamental cabbage and bean, wheat, rice, and mushroom is 0.2 mg kg⁻¹ and in potato and other root plants is 0.1 mg kg⁻¹ (Rezai, 2004).

Based on the researches done and comparison of toxic effect in different plant organs, this study can be an applied research for improving and producing plant varieties with lower ability of Cd absorption. In this research, the effect of different levels of Cd on the quality of cherry tomato, an ornamental and edible plant, were investigated.

MATERIALS AND METHODS

This research was conducted in Isfahan Center for Research of Agricultural Science and Natural Resources in 3 stages (germination, greenhouse, and laboratory) during one year.

Germination Stage

Tomato seed (*Lycopersicon esculentum* Mill. var. Cerasiform) are very resistance to Mosaic virus, vertislom, dejection. They have pome fruits with weight of 150-170g. These seeds were disinfected by 50% MnO₄ in 30° C and 70% humidity. Then they were transported to seeding vases (these vases were sterilized by Sodium Hypochlorite) of pitmas. After 3 weeks, 3 leave germs were ready to transport to solution in hydroponic environment.

Greenhouse Stage

Based on this fact that this research was done in hydroponic culture environment, 9 replications were considered for each treatment in 3 harvesting stages in 54 vases. The nutrition solution cycling was done by using 6 supplies in distinguish time and speed. For preparing Hoagland nutrition solution (Gamburg and Walter, 1975), microelements were used in form of HBO₃, MnSO₄, CuSO₄, ZnSO₄, Mo and macroelements were used in form of CaNO₃, KNO₃, MgSO₄, K₂HPO₄FeSO₄.

By using CdCl₂, 21/2 H₂O with molecule mass of 228.34 and 99.9 % purity. Treatments were studied in their final volume. After preparing nutrition solution, germs were transported from seeding vases to the vases containing substra perlite. After planting 3 leaves germs, their pH of nutrition solution, temperature, green house humidity, and amount of imported/exported solution were measured.

Laboratory Stage

Stage 1: Preparing Solutions with Solvable and Starch (Kochert, 1978)

In this stage for detachment of solvable and starch, 0.05 g of dry plant material from aerial parts was used in 3 replications in two harvesting stages: vegetative and flowering. The dried plant materials added to the jar containing 10 ml ethylic alcohol (80%). The refrigerant was put in the Arleen and they were heated in 100°C hot bath. Then the content of the Arleen were cooled and filterized. After that their volume was increased to 100ml. The content of filter paper containing starch in ethanol was put in 75°Coven. Finally they were poured into a basher and boiled in hot distilled water for 15 minutes. 5 ml of Ba (OH)₃ (0.3 N) and 5 ml of ZnSO₄ (5%) were added to the volume of filterized solution containing solvable sugar. After 10 minutes centrifuging by G rotation, 1000 ml supernatant in a sterilized water balloon was increased to 100 ml volumes. Finally, the residual of pipe containing ZnSO₄ and pigments were discarded.

Stage 2: Adding Indicator

For measuring and determining sugar, phenol indicator (5%) and H₂SO₄ were used. The 2 ml of prepared solutions was put in separate pipes and for each pipe 1 ml phenol (5%) and 5 ml H₂SO₄ were added. When cooling was done, if sugar was in the solution, the environment would be changed to yellow color. The absorption of each solution in the wave length of 485 nm was read and the amount of sugar samples was investigated by using standard curve based on g kg⁻¹. For determining density of solvable and starch of samples, solutions with concentrations of 0, 1, 2, 5, 10, 20, 50 and 100 ml L⁻¹ in glucose were prepared (control sugar). The whole process was conducted and repeated in 2 ml of each sample. Then by using written absorption numbers, the standard curve was calculated based on this formula $C = ABS \times 0.0134 + 0.00157$ and the amounts of sugars were measured.

(C=sugar concentration, ABS= the amount of absorption in wave length of 485 nm)

Measuring Protein Amount Based on Kjeldahl Method (ISRC, 1990)

Measuring protein amount was conducted based on the measurement of total nitrogen (Ter-racing method) after distillation. First, 0.25 g of plant dry material powder (aerial and digestive parts) was put in the pipes containings salicylic acid and H₂O₂. Then 2 ml salicylic acid and 1ml H₂O₂ were added again to pipes for discoloring samples. Then the samples volumes were increased to 50 cc in balloon. After that, 5 ml of extract was pipette and transported to distillation balloon. The amount of 2 ml of NaOH (12.5 mol L⁻¹) was added and the balloon was heated in water bath for 3 minutes. The solution was absorbed in 10 ml HBO₃ containing 10 drops of indicator. Ultimately, HBO₃ solution containing NH₃ was titrated with H₂SO₄ 0.005 mol. This process changed the color of the solution from blue to pink.

Boric acid is a weak acid and it releases NH₃ in adjucent to strong acid. The percentage of nitrogen absorbtion (NA) in dry plant samples was calculated based on the following formula:

$$NA \text{ percentage} = 0.56 \times t \times (a-b) \times v/w \times 100/DM$$

(t = the concentration of acid in titration, a= the amount of acid used in sample, b= the amount of acid used in control ml, v = the volume of digestion ml, w=the weight of plant sample g, DM= the percentage of dry plant material)

The total percentage of plant nitrogen absorbtion is calculated based on g kg⁻¹ and for converting nitrogen absorbtion into protein, it needs F special coefficient.

The Measurement of Elements (Moral, 1996)

Plant samples (1 g of each sample) were digested by mixture of H₂O:HNO₃ in ratio of 9:1. Then the volume of samples increased to 25 ml. For measuring Cd, I.C.P system and standard curve were used based on mg L⁻¹.

Statistical Analysis

The total tests of Cd were conducted randomly in 3 stages and 9 replications in p≤0.01 probability level. The results were analyzed through SPSS (version=14.00) and Excel soft wares.

RESULTS AND DISCUSSION

In this study the effects of Cd on *Lycopersicum esculentum* Mill. var. Cerasiform was investigated in six treatments (0, 0.1, 0.3, 0.9, 2.7 and 10 μm) with 3 replications in hydroponic environment. The study was conducted in 3 harvesting stages: vegetative, flowering and full product. Firstly, 18 vases (3 replications for 6 treatments) were used for investigating the physiological and biochemical parameters. The analysis of the results and statistical data of these two stages was conducted by ANOVA and LSD in RCBD. At the third stage, the amounts of accumulated Cd in root, leaf, and fruit were measured and compared to allowed level of Cd in the agricultural products.

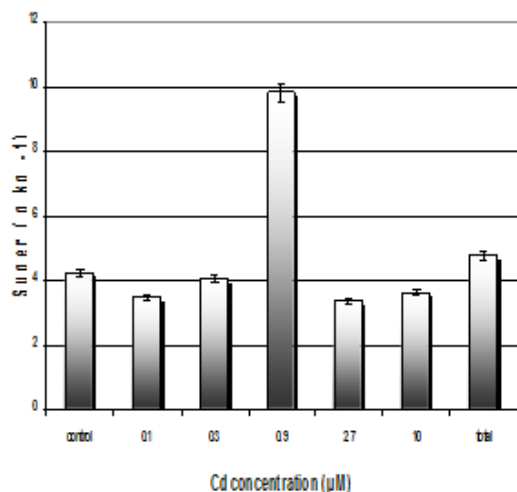


Fig. 1. The effect of different levels of Cd on soluble sugar in vegetative stage.

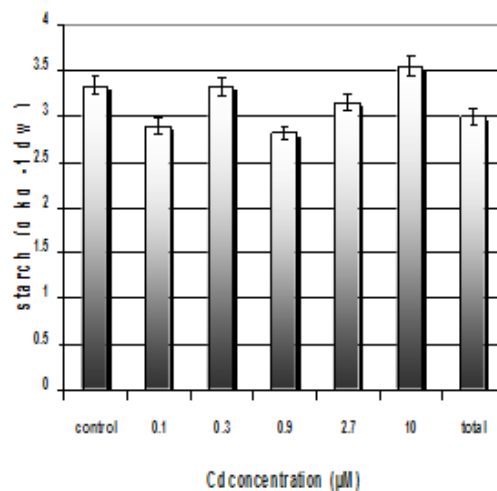


Fig. 2. The effect of different levels of Cd on starch in vegetative stage.

In all stages, the increase of Cd concentration was shown compared to the control. Finally, the results and statistical data of different parameters such as growth, physiological and biochemical changes were compared to each other.

The Effect of Cd on Solvable Sugar and Starch

The results obtained from ANOVA revealed that there isn't significant differences among six treatments in terms of solvable sugars in vegetative stage ($p \leq 0.05$) and starch ($p \leq 0.05$) in dry plant weight (Fig. 1 and 2). After 40 days (at the end of flowering stage), Cd was demonstrated as a blockage element in plant metabolism, and it caused solvable sugar reduction in 7.56 g kg^{-1} compared to the control plants (3.88 g kg^{-1}) in 10 µM treatment. The 49% reduction showed significant differences ($p \leq 0.001$) in all Cd concentrations compared to the control. On the other hand, in case of negative acceleration of growth by increasing Cd in the medium, amount of starch ($5.19 \text{ g kg}^{-1} \text{ DW}$ in control) maximized to $8.11 \text{ g kg}^{-1} \text{ DW}$ in 2.7 µM Cd concentration. LSD test demonstrated differences ($p \leq 0.05$) between control and concentrations of $0.3, 0.9, 2.7$ and 10 µM . Figs 3 and 4 show the means of solvable sugar and starch in dry weight in flowering stage.

By Cd treatment, carbon cycle is blocked. Cd has a strong effect on rubisco and balance between CO_2 and O_2 . The effect of Cd on rubisco activity is an important factor due to its reaction with SH group. On the other hand, when Cd stresses starts, it has effect on calvin cycle and its en-

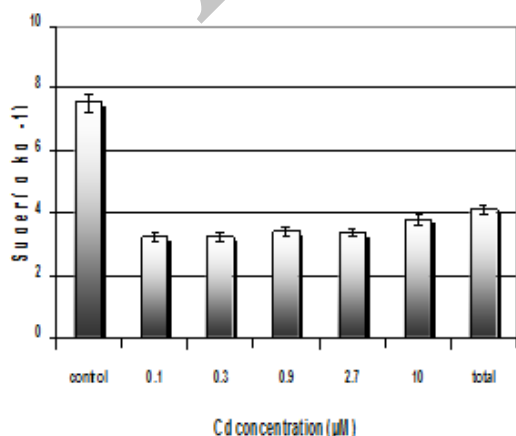


Fig. 3. The effect of different levels of Cd on soluble sugar in flowering stage.

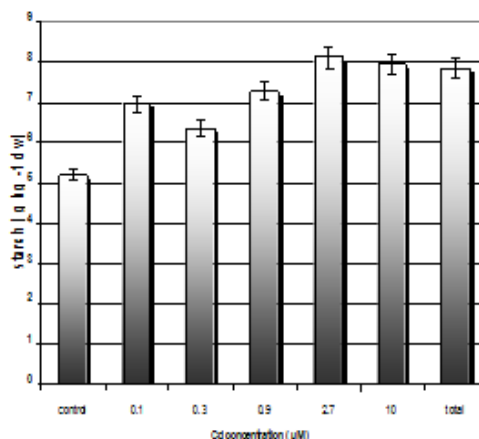


Fig. 4. The effect of different levels of Cd on starch in flowering stage.

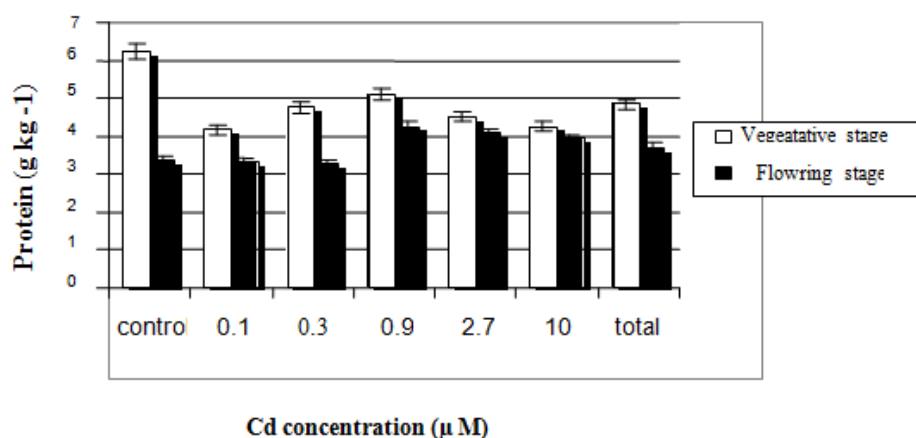


Fig. 5. The effect of different levels of Cd on plant protein in vegetative and flowering stages.

zymes (Vassilev and Yorrdanor, 1997).

The Effect of Cd on The Amount of Plant Protein

The results of the analysis showed that in vegetative stage the maximum amount of protein ($6.23 \pm 0.62 \text{ g kg}^{-1}$) in control was reduced to 4.18 g kg^{-1} (in $0.1 \mu\text{M}$ Cd concentration). This reduction in all treatments was due to gradual increase of Cd concentration ($p \leq 0.01$).

By starting of treatments the amount of protein accidentally reduced to 33% in $0.1 \mu\text{M}$ Cd concentrations and this gradual reduction could also be seen in other Cd treatments. On the contrary, the analysis of statistical data showed that the amount of protein increased in flowering stage compared to control. This increase in heavy metal stress environment affected on protein synthase enzymes (Prasad, 1997; Kneer and Zink, 1992). In Fig. 5, the amount of protein in aerial organs shows significant difference.

Cd Accumulation in Root, Leaf, and Fruit

Plant species and varieties are very different in the ability of absorption, accumulation, and resistance to heavy metals (Alloway, 1990). The previous studies on heavy metal concentration indicated that plant species are important to Cd treatment and Cd accumulates in roots. Cd accumulation in other organs is related to Cd accumulation in root (Jana *et al.*, 1987). There was a significant difference between control and different Cd treatments ($p \leq 0.001$). There was a correlation between available Cd and its concentration in different plant organs. In high Cd treatment, higher Cd accumulation $0.0383, 0.0206, 0.0201 \text{ mg kg}^{-1} \text{ dw}$ was seen in root, leaf, and fruit, respectively.

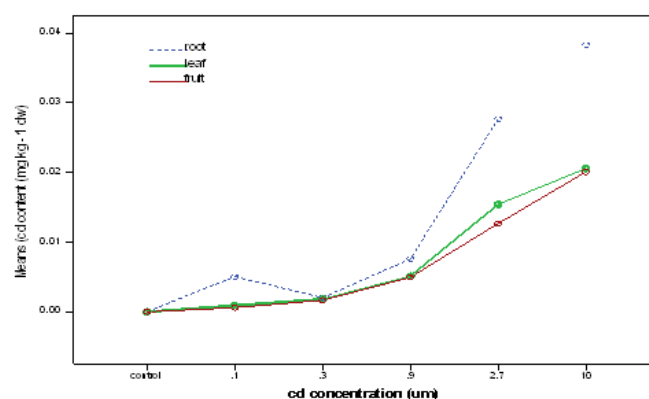


Fig. 6. The comparison of the amount of Cd accumulation in different organs.

GLM variance test of aerial (leaf – fruit) and underground organs exhibited high concentration of Cd in root in 10 μ m (Fig. 6). Chauderi *et al.* (1995) reported that 12-18 percent Cd transport from aerial organs of cereals to their seeds. Some researchers classified high concentrations of Cd in order of roots, leaves, seeds, and reservation organs. There was a correlation between available Cd and its concentration in different plant organs. The plant grown in soil contaminated with Cd had high accumulation in their root, stem, and leaves. In the polluted calcareous soil, the ratio of Cd in leaves and fruit of plant was 30 – 60 mg kg⁻¹dw. In plants grown in accumulated soil (100 ppm) the specific coefficient of Cd accumulation in fruits (fruit/soil=1.10) was due to high speed of Cd transport from the soil to aerial organs of plant.

CONCLUSION

This study reveals that the increase of Cd concentration causes the 49% reduction of sugar solution in 10 μ m Cd treatment. On the other hand, the gradual increase of Cd in medium causes the increase of starch, reduction of photosynthesis and blockage of carbon cycle enzymes. Furthermore, in heavy metal stress condition in vegetative stage, Cd has negative effects on the protein amount of total treatments in $p \leq 0.01$ signified level. When treatment starts, the amount of protein reduced 33% in 0.1 μ m Cd concentration and this reduction can be seen in other treatments. In contrast, in flowering stage the amount of protein increased compared to control. The GLM variance test of aerial and underground plant organs showed that the high level of Cd concentration in root in 10 μ m Cd concentration. This increasing process was seen in root, leaf, and fruit. The accumulation of Cd expresses high speed of Cd transport from the soil to upper organs of cherry tomato, and this process reduces the amount of solvable sugar and protein.

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