

Phytotoxicity of chromium (III and VI) on tomato (*Lycopersicum esculentum* L.) grown in sand culture

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Research Article

Keywords: Chromium, tomato, sand culture, growth, oxidative stress, nutrient status

Posted Date: March 24th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-2696066/v1>

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Abstract

Chromium (Cr), an element naturally occurring in earth's crust, and a known toxic heavy metal, causes deleterious effects on plants when present in excessive amounts. The present study is aimed to evaluate the effects of both trivalent and hexavalent Cr separately on tomato plants. For this, a sand culture glasshouse experiment was conducted with 0.1 mM Cr³⁺, 0.2 mM Cr³⁺, 0.1 mM Cr⁶⁺, 0.2 mM Cr⁶⁺ doses to access the Cr accumulation pattern and study its effects on growth, morphology, photosynthetic pigments, nutrient status and oxidative stress parameters in tomato. Chromium toxicity resulted in growth depression and reduction in chlorophyll content of plants. Cr caused wilting and necrosis of especially old leaves. Cr was mainly found to be accumulated in roots, and also caused hindrance in the translocation of micronutrients to upper parts of the plants. Cr toxicity caused oxidative stress which was reflected in the form of increased levels of lipid peroxidation and anti oxidative enzymes. Plants did not bear any fruits at 0.2 mM Cr³⁺ and 0.2 mM Cr⁶⁺ treatments. Among both forms of Cr (3+ and 6+) tested, Cr⁶⁺ clearly proved to be more toxic for growth and metabolism of plants. However, at many places, 0.2 mM Cr³⁺ showed more toxicity than 0.1 mM Cr⁶⁺ stating that higher doses of Cr³⁺ are also very harmful to tomato plants.

Introduction

Environmental pollution due to toxic heavy metals has accelerated tremendously in the past few decades. When these hazardous pollutant elements are taken up by plants, produce lethal effects, which directly or indirectly, affects human health. One such toxic heavy metal is chromium, which causes deleterious effects on plant growth and development (Shanker et al., 2005). Toxicity of chromium to plants is mainly dependent on its valence state. It is seen that Cr primarily exists in two stable oxidation states, i.e. +III and +VI, out of which Cr(VI) is highly toxic and more harmful than Cr(III) (Becquer et al., 2003). Cr(VI) is a strong oxidant, which induces high generation of reactive oxygen species, and accounts for its very high toxicity (Shanker et al., 2004).

Previous studies have showed that high concentrations of Cr, severely hampered the growth and yield of plants (Chidambaran et al., 2009). Cr absorbed by plants is mainly accumulated in roots and is not very readily translocated to upper parts (Singh et al., 2004). Toxic impact of Cr in plants mainly includes reduced growth, alteration in enzymatic activities, ultrastructural modifications and changes in its nutrient status etc (Han et al., 2004; Panda and Patra, 2000; Panda and Choudhury, 2005). Cr effects on plant pigments, photosynthetic parameters, enzymatic and non-enzymatic antioxidative system have been reported in many plants (Tang et al., 2014; Oliveira, 2012; Samantaray et al., 2001; Rahman et al., 2010; Rai et al., 2004; Ma et al., 2016; Maiti et al., 2012; Sharma et al., 2020).

It is reported that trivalent and hexavalent Cr may interconvert in soil as reported by James and Barlett, 1983; and Ottabong 1989a,b; and since soil immobilize both these forms of chromium (Stollen- Werk and Grove 1985; Ottabong 1989), it is difficult to access separately the impact of these two forms on plants (Samantaray et al., 1998). Therefore, in the present study an attempt has been made to study the

effects of these 2 forms of Cr i.e. III and VI separately on *Lycopersicum esculentum*, an important cultivated crop across the globe.

Materials And Methods

Plant material and experimental set up

Tomato (*Lycopersicum esculentum* L. cv Navodaya) plants were grown in refined sand in a glass house at an ambient temperature (25°–30°C). Plants were grown in polyethylene containers of 10 L capacity and subjected to different doses of Chromium (III and VI forms). A total of 5 treatments were maintained i.e. control, 0.1 mM Cr³⁺, 0.2 mM Cr³⁺, 0.1 mM Cr⁶⁺, 0.2 mM Cr⁶⁺. Cr(VI) was supplied as potassium dichromate and Cr (III) was supplied as chromium trioxide in nutrient solution. Plants were given a daily supply of nutrient solution whose composition was as follows: 4 mM KNO₃; 4 mM Ca(NO₃)₂; 2 mM MgSO₄; 1.5 mM NaH₂PO₄; 0.1 mM Fe–EDTA; 0.1 mM NaCl; 30 µM H₃BO₃; 1 µM CuSO₄; 1µM ZnSO₄; 0.2 µM Na₂MoO₄; 0.1µM CoSO₄; and 0.1 µM NiSO₄ (Hewitt, 1966). The pH of the nutrient solution was maintained at 6.7 ± 0.2. Initially the seedlings were maintained in complete nutrient solution for 17days. On 18th day, plants were separated into five lots. One lot was allowed to grow with full nutrient solution to serve as control. The other four lots received solutions of 0.1 mM Cr³⁺, 0.2 mM Cr³⁺, 0.1 mM Cr⁶⁺, 0.2 mM Cr⁶⁺ along with basal nutrient solution. On Sundays, each pot was flushed with distilled water to remove accumulated salts and root exudates. For each treatment 3 replicates were taken. The pots were arranged in randomized block design. The visible symptoms of Cr toxicity were recorded periodically. Plants were sampled for morphological and biochemical observations at 14 days after metal supply (DAS). Estimation of Cr concentration and micronutrients was performed in plant parts at 62 DAS (at harvest).

Morphological observations and dry matter yield

The root and shoot lengths together with their fresh weights of chromium treated and untreated (control) tomato plants were measured after washing and rinsing plants with tap and deionised distilled water respectively. Dry weight of roots and shoots were determined after oven drying at 70⁰C for 48 hours.

Morphological observations and dry matter yieldTotal chlorophyll, chlorophyll a and chlorophyll b contents were measured according to Arnon (1949). Chlorophyll contents were expressed in terms of mg chlorophyll present / g fresh weight of tissue. Carotenoid contents were estimated according to method of Duxbury and Yentsch (1956).

Lipid peroxidation

Lipid peroxidation in terms of malondialdehyde (MDA) was determined to access the membrane damage in plants. For the measurement of lipid peroxidation, TBA (Thiobarbituric acid) test was used to measure MDA level as an end product of lipid peroxidation (Heath and Packer, 1968).

Anti oxidative enzymes

Activity of catalase (CAT) enzyme was estimated according to Euler and Josephson (1927). Peroxidase (POD) activity was estimated according to Luck, 1963. Superoxide dismutase (SOD) activity was assayed by the method of Beauchamp and Fridovich (1971) by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT).

Chromium and micronutrients' concentration

Oven dried plant parts were digested for estimation of Cr and micronutrients' concentration using protocol of Piper (1942).

Statistical analysis

The experiment was conducted in a completely randomized design (CRD) with 3 replications. The data were analyzed by One Way ANOVA using software program Sigmastats 3.5. It was followed by comparison of mean values using Holm Sidak method at $p \leq 0.05$.

Results

1. Growth and visible symptoms

Chromium toxicity resulted in growth depression of tomato plants (table 1). Shoot and root length decreased on Cr application. Growth reduction was more in Cr^{6+} treated plants than Cr^{3+} plants. Also, decrease in root length was more pronounced. Likewise, plant fresh weight and dry weight also suffered reduction in the above similar manner. After 3-4 days of metal exposure, depression in growth was seen in 0.2 mM Cr^{6+} and, after 10 to 12 days of metal supply growth depression was apparent in nearly all Cr treated plants. In 0.2 mM Cr^{6+} treatment, tomato plants were not able to survive after 49 days of metal supply. The symptoms of Cr stress appeared on older leaves as chlorosis, which gradually spread to upper leaves. Affected leaves turned yellow, became wilted and due to loss of turgor hung down from petioles. Lamina of leaves became permanently wilted, necrotic, dry and shed.

2. Photosynthetic pigments

The chlorophyll a content decreased to 43% in case of Cr^{3+} (0.2 mM), whereas the drop was 58.4% in Cr^{6+} (0.2 mM). Chlorophyll b dropped only 9.9% and 39% in above cases as compared to control (table 2).

3. Lipid peroxidation

Lipid peroxidation increased to 43.245 nmol MDA/100 mg fw in leaves of Cr^{3+} (0.2 mM) plants from 26.97 in control. However, in case of Cr^{6+} (0.2mM), it rose to 54.405 which is nearly 102% increase in its value (table 3).

4. Anti oxidative enzymes

Catalase activity mostly decreased on Cr application. However, a mild increase in its value was seen in leaves at 0.1 mM Cr³⁺. Peroxidase and superoxide dismutase increased in leaves at most Cr applied doses (table 3).

5. Chromium concentration

At 62 days after metal supply, Cr was found to be accumulated in roots mainly. With increase in Cr supply, all parts of plant accumulated Cr in their tissue in increasing order (fig. 1).

6. Micronutrient concentration

Mn, Cu and Fe reduced in Cr treated plants, however Cu and Fe enhanced in roots of 0.1 mM Cr⁶⁺ treated plants (fig. 2). Zn didn't show any particular trend in Cr toxicity. Fruits of Cr³⁺ and Cr⁶⁺ treated plants showed reduced concentration of Fe, Cu, and Mn as compared to control. However, Zn showed marginal enhancement (fig. 3).

Discussion

As far as physiology of plant is concerned, Cr does not have any known biological role (Reale et al., 2016). Moreover, it is commonly perceived that extreme Cr levels in plant tissues may disrupt several morphological, physiological and biochemical processes (UdDin et al., 2015; Kamran et al., 2017).

In the present study, Cr (both in +3 and +6 forms) reduced growth of tomato plants as evident from reduction in all its studied parameters which is in accordance with earlier reports on Cr toxicity in plants (Shanker et al., 2005) where reduction in germination; leaf, root and shoot growth; yield and dry matter production was seen. As compared to other heavy metals, the mobility of Cr in the plant roots is low, which eventually leads to the higher concentration of Cr in the roots as compared to above ground parts (sometimes 100 times higher in roots than in the shoots) (Shanker et al., 2005; Gupta et al., 2006). Decreased root growth can also be attributed to inhibition of root cell division or root elongation under Cr toxicity. Roots in contact with Cr, is unable to properly absorb water and eventually collapses (Barcelo et al., 1986).

The symptoms of Cr toxicity observed in the present study are similar to symptoms seen in cabbage (Pandey and Sharma, 2003), green gram (Sinha et al., 2006) and ground nut (Gopal and Sharma, 2014). These observed symptoms are however, in contrast with observations of Pedreno et al., 1997 where it was reported that young leaves in tomato were more affected due to Cr toxicity.

Cr (+3 and +6 forms) treatments, showed significant reduction in pigment concentration which is in accordance with earlier reports (Rai et al., 2014; Rajendran et al., 2019; Van Assche and Clijsters, 1983; Zeid 2001; Shanker 2003). This reduction in pigment levels could be due to the inhibition of chlorophyll biosynthesis under Cr stress (Lushchak, 2011; Shama et al., 2019). It is seen that increased concentration

of Cr generally leads to the deterioration of the chlorophyll content or disrupted chlorophyll biosynthesis in many plants (Valko et al., 2006; Chandra and Kulshreshtha, 2004). Reason for decline in chlorophyll contents may also be due to the increased activity of enzymes like chlorophyllase, as well deficiency and reduced translocation of important nutrients in plants in presence of heavy metal toxicity (Khan et al., 2016; Shakoor et al., 2014). In the current study, it was also observed that chlorophyll a was more affected than chlorophyll b in both Cr (+3 and +6) forms. This is in concurrence with reports of Bishnoi et al. 1993 where more pronounced effects of Cr were seen on PS I than PSII activity in isolated chloroplasts. However, contrasting results were observed by Davies et al. (2002) who noticed that Cr inhibited photosynthetic process by targeting PSII. Photosystem I consists largely of chlorophyll a molecules and a decrease in chlorophyll a content may indicate a reduction in size of peripheral part of antenna complex in tomato leaves.

Heavy metals have the tendency to generate ROS (reactive oxygen species) if they exceed permissible limits (Shahzad et al., 2018; Duquesnoy et al., 2010; Marquez- Garcia et al., 2012; Korpe and Aras, 2011). Reports advocate that the enhanced generation of ROS in plants under Cr toxicity leads to oxidative burst which causes damage to DNA, lipids, pigments, proteins and stimulating the process of lipid peroxidation (Choudhury and Panda, 2005; Ullah et al., 2019). Several reports have been documented where severe increase in ROS was observed (Ali et al., 2015; Anjum et al., 2015; Fahad et al., 2019), with increased malondialdehyde (MDA) contents under Cr toxicity (Adrees et al., 2015). Cr (+3 and +6) application showed increased lipid peroxidation (in the form of MDA levels), in leaves of tomato, reflecting its role in oxidative stress.

To combat oxidative stress, various antioxidants and anti-oxidative enzymes in plants come into play. Among anti-oxidative enzymes, superoxide dismutase (SOD) is considered as a first line of defense against various stresses (Shahzad et al., 2018; Gill and Tuteja, 2010). Dismutation of superoxide ion (one of the reactive oxygen species) is catalyzed by the SOD enzyme, which is localized in almost all the cellular compartments, leading to the production of hydrogen peroxide and release of oxygen (Gill and Tuteja, 2010), thereby giving relief to the plant cell by converting ROS to less toxic products. In present case also, most of the applied Cr treatments led to an increase in SOD levels showing its role in combating oxidative stress. Excess H_2O_2 generated due to SOD activity is detoxified by catalase (CAT, an important antioxidant enzyme). CAT is responsible for dismutation of H_2O_2 into O_2 and H_2O , rendering H_2O_2 less harmful (Mmamdi et al., 2010). However, here, a significant decline in CAT activity at higher Cr dose suggests that this enzyme is not involved in protection of tomato plants against Cr toxicity. On the other hand, increased activity of peroxidase (POD) and SOD enzymes on Cr application suggests their protective role in Cr toxicity.

In the present study, the higher accumulation of Cr in roots of the tomato plants might be attributed to the sequestration of Cr in the vacuoles of root cells, which acts as a protective mechanism (Mangabeira et al., 2011) and provides a natural tolerance to plants towards Cr toxicity (Shanker et al., 2004). The translocation of Cr from the roots to the aerial shoots is also very limited and it depends on the chemical form of Cr inside the tissue (Shahid et al., 2017). In plant tissues, the Cr (VI) is converted to Cr (III) which

has the tendency to bind to the cell walls, which hinders the further transport of Cr within plant tissues (Kabata-Pendias and Szteke, 2015).

Heavy metal stress affects nutrient uptake in plants by interacting with other essential minerals. In the present study, Cr application mostly reduced the concentration of micronutrients studied. Except Zn, rest of the micronutrients studied, showed decrease in their levels in tomato fruits with increasing doses of Cr. Higher levels of both forms of chromium (3+ and 6+) did not bear any fruit, thus hampering the reproduction of plant. Also, micronutrients were mainly accumulated in roots and were not able to translocate to upper portions of plants under Cr application. Excessive Cr had been observed to reduce the uptake of essential minerals like iron (Fe), magnesium (Mg), phosphorus (P) and calcium (Ca) by masking the sorption sites and forming insoluble complexes (Kabata-Pendias and Szteke, 2015; Osu and Onyema, 2016). Cr is known to disturb the nutrient balance of plant (Dube et al., 2003). A similar decrease in the uptake of micronutrients like Zn, Cu, Fe, Mn and macronutrients like potassium (K), P and nitrogen (N) had been noticed in the paddy plants (*Oryza sativa* L.) under excessive Cr exposure (Sundaramoorthy et al., 2010).

Among both forms of Cr (3+ and 6+) tested for its effects on tomato, Cr⁶⁺ clearly proved to be more toxic for growth and metabolism of plant. All the parameters tested under the purview of Cr⁶⁺ showed more deleterious effect. However, at many places 0.2 mM Cr³⁺ showed more toxicity than 0.1 mM Cr⁶⁺ stating that higher doses of Cr³⁺ are also very harmful for plants.

Declarations

Acknowledgements:

Author is grateful to ICAR AICRP (MSPE), University of Lucknow for providing necessary facilities for the study.

Competing interests: Authors declare no competing interests.

Authors' contributions: Not applicable

Funding: No funding received.

Availability of data and materials : Not applicable

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Tables

Table 1. Effect of different treatments of chromium (3+ and 6+) on growth and morphological parameters of tomato.

Parameters	Control	0.1 mM Cr ³⁺	0.2 mM Cr ³⁺	0.1 mM Cr ⁶⁺	0.2 mM Cr ⁶⁺
Shoot length (cm)	61.18±0.6	48.98±0.6*	35.10±1.1*	43.20±0.7*	33.47±0.3*
Root length (cm)	18.37±0.7	14.40±1.0*	9.71±0.4*	10.03±0.3*	8.07±0.2*
Shoot FW (g)	62.83±0.1	17.43±0.5*	7.16±0.1*	14.20±0.1*	5.41±0.3*
Shoot DW (g)	6.13±0.3	2.29±0.0*	1.12±0.05*	2.02±0.02*	1.12±0.06*
Root FW (g)	9.53±0.3	2.52±0.1*	2.05±0.3*	2.44±0.1*	1.73±0.1*
Root DW (g)	1.10±0.01	0.63±0.04*	0.50±0.01*	0.55±0.0*	0.205±0.0*

FW= Fresh weight; DW= Dry weight

Values are means of three replicates ± S.E.*Data significant at p < 0.05. Multiple comparisons Vs Control group (Holm Sidak method) Overall significance level = 0.05

Table 2. Effect of different treatments of chromium (3+ and 6+) on photosynthetic pigments of tomato.

Parameters	Control	0.1 mM Cr ³⁺	0.2 mM Cr ³⁺	0.1 mM Cr ⁶⁺	0.2 mM Cr ⁶⁺
Chl a (mg/g FW)	2.660±0.021	1.516±0.003*	1.515±0.610*	1.242±0.006*	1.107±0.003*
Chl b (mg/g FW)	1.565±0.040	1.564±0.002	1.410±0.041*	1.298±0.006*	0.953±0.004*
Total Chl (mg/g FW)	4.210±0.061	3.077±0.020*	2.922±0.006*	2.513±0.059*	2.057±0.003*
Carotenoids (mg/g FW)	0.965±0.006	0.860±0.041*	0.825±0.002*	0.710±0.006*	0.585±0.001*

Chl= Chlorophyll; FW= Fresh weight

Values are means of three replicates ± S.E.*Data significant at p < 0.05. Multiple comparisons Vs Control group (Holm Sidak method) Overall significance level = 0.05

Table 3. Effect of different treatments of chromium (3+ and 6+) on lipid peroxidation and anti oxidative enzymes in tomato leaves.

Parameters	Control	0.1 mM Cr ³⁺	0.2 mM Cr ³⁺	0.1 mM Cr ⁶⁺	0.2 mM Cr ⁶⁺
Lipid peroxidation (n mol MDA/ 100 mg FW)	26.36±2.19	35.07±2.81*	43.18±0.61*	39.51±0.46*	54.67±0.59*
Catalase (μM H ₂ O ₂ decomposed/ mg protein)	580.9±2.7	589.7±5.05	500.87±1.6*	530.2±2.02*	421.2±2.97*
Peroxidase (ΔOD/ mg protein)	0.616±0.04	0.724±0.02*	0.808±0.21*	0.734±0.03*	0.77±0.0*
Superoxide dismutase (EU/ mg protein)	5.664±0.031	5.424±0.23	6.048±0.04*	6.528±0.29*	6.208±0.02*

Values are means of three replicates ± S.E.*Data significant at p < 0.05. Multiple comparisons Vs Control group (Holm Sidak method) Overall significance level = 0.05

Figures

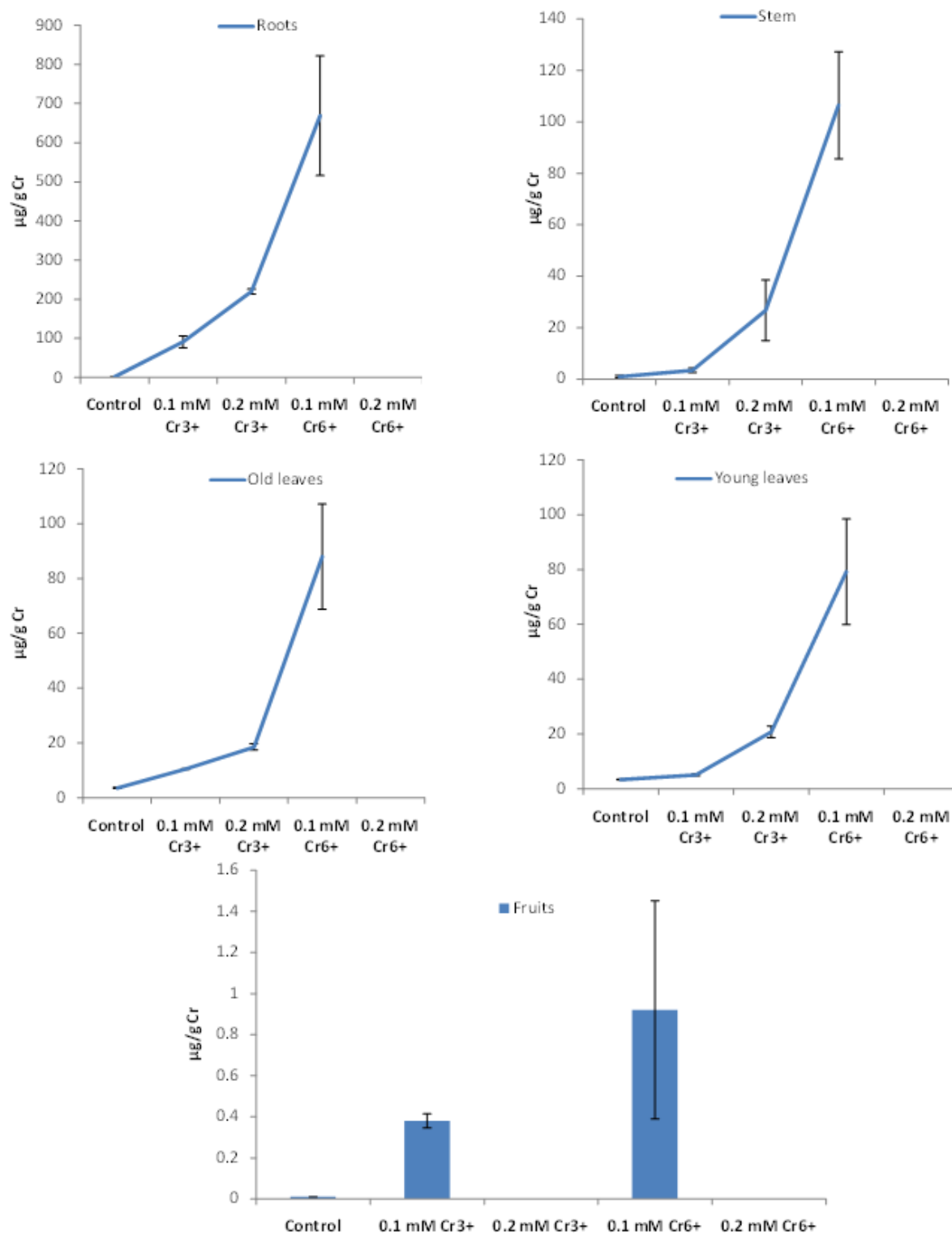


Figure 1

Graph showing concentration of Cr in plant parts of tomato at various doses of applied Cr (+3 and +6).

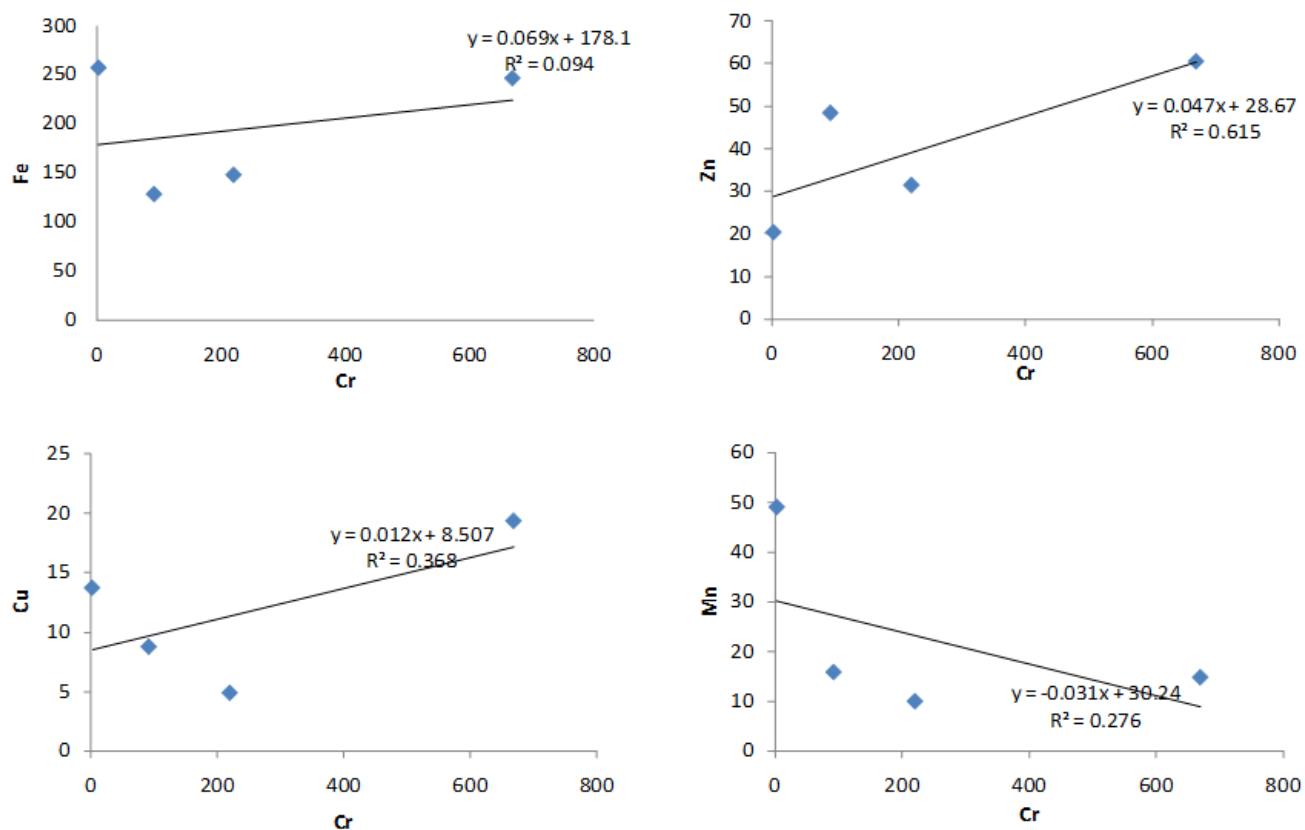


Figure 2

Graph representing correlation between Cr concentration and micronutrients' concentration in roots of tomato at various doses of applied Cr (+3 and +6).

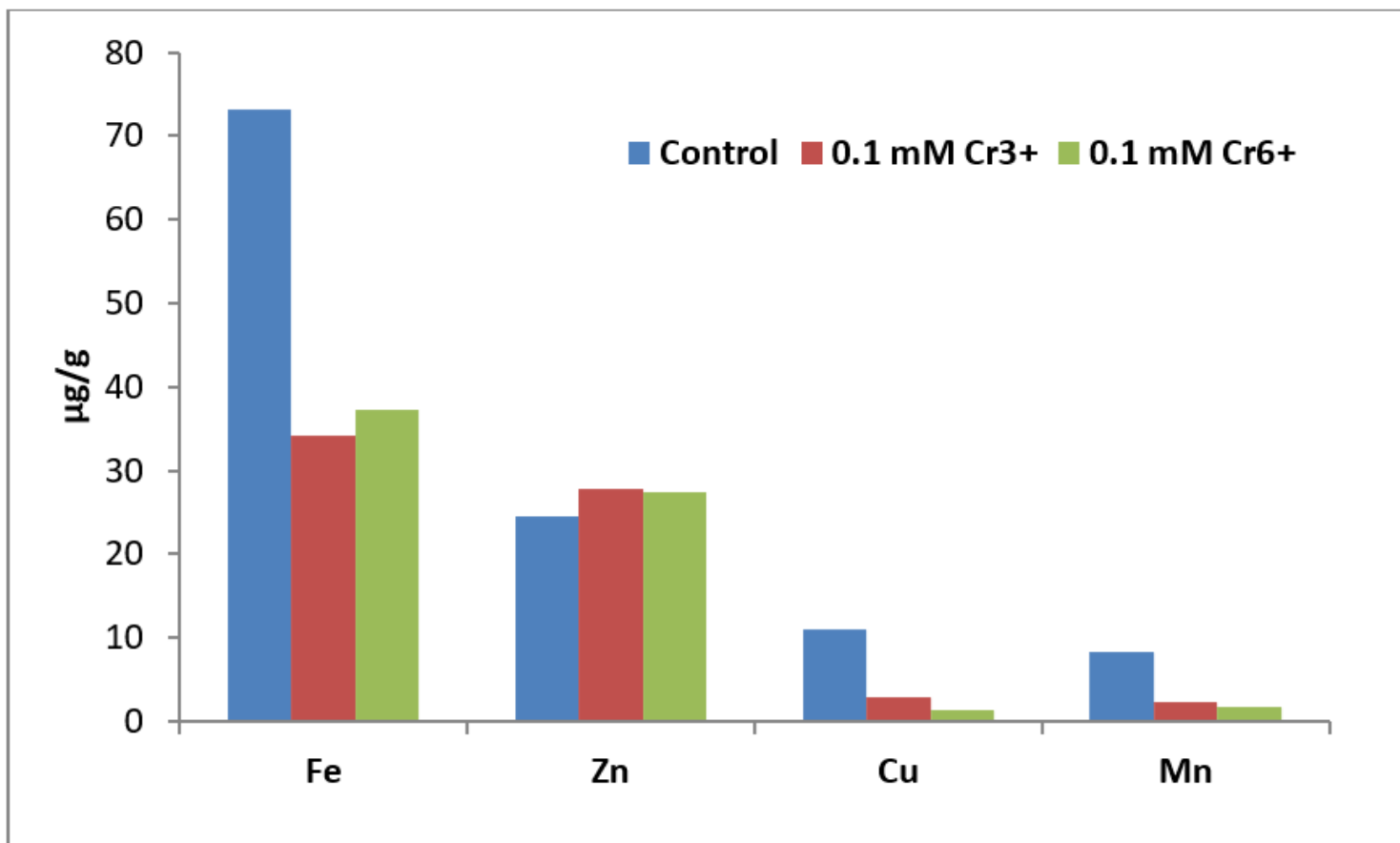


Figure 3

Status of micronutrients in tomato fruits under different treatments of chromium (3+ and 6+).

Plants treated with 0.2 mM Cr³⁺ and 0.2 mM Cr⁶⁺ did not bear any fruit.