

*Full Length Research Paper*

# Effects of high concentration of chromium stress on physiological and biochemical characters and accumulation of chromium in tea plant (*Camellia sinensis* L.)

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**We studied the effects of high concentration of chromium (Cr) stress on physiological and biochemical characters and accumulation of Cr in Pingyang Tezao tea [*Camellia sinensis* (L) O. Kütze 'Pingyangtezao'] through a pot experiment. The results show that the indicators of photosynthesis were all suppressed with increasing Cr stress, and antioxidation and protection systems were destroyed. We also discovered that the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) decreased, while the content of proline (Pro) and malondialdehyde (MDA) and relative conductivities increased. The accumulating Cr in all organs had a positive correlation with Cr concentration. Deposition of Cr in organs was in the following order: stubble > root > leave > stem > branch ≈ new stem (a bud and two leaves). We conclude that high concentration of Cr stress had strong inhibition and damage to the normal metabolism and cell implement structure, as well as on the function of tea plant.**

**Key words:** Tea plant, chromium, physiological and biochemical characters, accumulation.

## INTRODUCTION

In recent years, heavy metal contamination as a result of electroplating, chrome plating, leather tanning, textile dyeing, batteries, paints and waste (Boominathan and Doran, 2003; Plunkett, 1987; Seiler et al., 1988; Donmez and Aksu, 2002; Kim et al., 2002), has become a serious problem all over the world. Heavy metal is a major threat to the environment, animals and humans due to their extreme toxicity (Kaewsarn and Yu, 2001; Emine and Yasar, 2006). Chromium (Cr) is a common contaminant and it is widespread in nature. The accumulation of soil Cr has a negative effect on tea plant. It not only affects tea production and quality, but also endangers our health through direct contact or from food chain to organisms. Therefore, it is very important in theory and practice to study Cr pollution. low concentration, it could promote plant growth; Cr is necessary for plant growth (Hu et al., 2010). In however, high concentration of Cr could poison

plant. In recent years, the content of Cr in tea is on the rise (Shi et al., 2006), which has severely inhibited the growth of tea. There are many studies showing that Cr stress has adverse effects on physio-biochemistry, absorption and accumulation of crops mainly rice, wheat and vegetables (Shi, 2004; Zhang, 1997; He et al., 2005; Hong et al., 1991; Xu et al., 2002). However, reports regarding effects of Cr on tea are few. Hence, in this study, we investigated the effects of Cr stress on some physiological indicators such as growth, photosynthesis, enzymatic activity, proline (Pro), malondialdehyde (MDA), cell membrane permeability (CMP) and accumulation in Pingyang Tezao tea [*Camellia sinensis* (L) O. Kütze 'Pingyangtezao'] through a pot cultivation experiment, to evaluate the adverse effects of Cr pollution on tea production and accumulation of Cr in tea plant.

## MATERIALS AND METHODS

### Tea plant and treatment

Pingyang Tezao tea were planted through a pot cultivation in which

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soil was sieved by 2 mm aperture sieve and then treated with Cr in concentrations of 0 mg·kg<sup>-1</sup>(Cr0), 300 mg·kg<sup>-1</sup>(Cr1), 400 mg·kg<sup>-1</sup>(Cr2), 500 mg·kg<sup>-1</sup>(Cr3) and 600 mg·kg<sup>-1</sup>(Cr4). Cr(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O (total molecular weight: 400.15; molecular weight of Cr: 51.9961) was the source of Cr and each treatment was repeated three times; every pot of soil was 7 kg (project in dry soil) and had four tea sprouts that were cultivated in accordance with a management level at the Tea Plantation Of Teaching Practice in Sichuan Agricultural University. The growth, photosynthetic indicators and the content of Cr in various organs were determined.

### Morphological and growth analysis

The indicators which contained the stem, root, stubble, leaves and branches of Pingyang Tezao tea were analysed. Every part of plant was weighed and tested for the growth of plants.

### Determination of chlorophyll

The content of chlorophyll of the second mature leaf of tea autumn shoots was detected with the method of extraction which mixed acetone, ethanol and water (the volume ratio was 4.5:4.5:1) and the value of optical-density was assayed at 645 and 663 nm. The chlorophyll content was computed by the method of Arnon formula.

### Measurement of photosynthetic physiological

The content of chlorophyll was determined with the method of spectrophotometry (Xu, 1995). We also studied the net photosynthesis rate (Pn), transpiration rate (Tr), intercellular CO<sub>2</sub> concentration (Ci) and stomatal conductance (Gs) of the second mature leaf of tea autumn shoots.

### Assessment of resistance indicators

The anti-oxidant ability of tea leaves was evaluated by measuring the activity of superoxide dismutase (SOD) with the colorimetric method of nitro-blue tetrazolium (NBT) (Xiong, 2003); peroxidase (POD) with the method of guaiacol oxidation (Xiong, 2003) and catalase (CAT) with the method of spectrophotometry (Wang, 2003). The oxidative stress was determined by measuring plasma MDA levels with the method of thiobarbituric acid (Zhao et al., 1991). CMP and the level of Pro, were also determined using sulfosalicylic acid (Wang, 2000) and conductivity meter of DDS-11A (Xiong, 2003), respectively.

### Determination of Cr

After 24 months, the whole plants were removed and washed and its new stem were steamed to de-enzyme them first, and then the stem, root, stubble, leaves and branches were dried in an oven at 80°C. The content of Cr was determined by the method of ICP-OES.

### Statistical analysis

Results were expressed as mean ± SD (standard deviation). The data were statistically analyzed using one-way ANOVA, followed by Duncan's multiple range tests to compare the means of all treatments.

## RESULTS

### Effects of high concentration of Cr on tea morphological characters and growth

The tea plants did not show the symptoms of disease until the sixth month in the high Cr stress. They showed different degrees of symptoms in different concentrations of Cr stress, and the roots suffered mostly, and then leaves and new stem. The leaves first displayed brown spots, and then scorched and dropped off. The number of new stems was small and the growth rate slowed down, even a part of them stopped growing and had scales emerging and most of the leaves became withered. The dry weight of the tea root, stubble, stem, branch and leaf reflected the growth of tea. As shown in Table 1, the dry weight of each organ decreased with increasing Cr concentration. The growth of tea organs were significantly different compared to the control group (Cr0). The growth of tea was inhibited by high concentration of Cr stress; the higher the concentration was, the stronger the inhibition of growth. In the highest concentration of 600 mg·kg<sup>-1</sup>, the inhibition was the strongest with the smallest growth rate, and the growth of root, stubble, stem, branch and leaf were reduced by 46.76, 52.19, 66.56, 69.06 and 66.67%, respectively, compared to the control group.

### Effects of high concentration of Cr on chlorophyll content

Chlorophyll is an important pigment and plays an important role in photosynthesis, and its content has a close correlation with photosynthesis (Liu et al., 2001). Table 2 shows that there was a significantly negative correlation between the content of chlorophyll a, chlorophyll b, total chlorophyll and Cr concentration in the high concentration of Cr stress, and the correlation coefficient were -0.97\*\*, -0.96\*\* and -0.99\*\*. Meanwhile, the composition of the chlorophyll also changed, and the ratio of chlorophyll a/b decreased with Cr concentration increase. The above results indicate that the degrees of damage of the chlorophyll a and b varied with different concentrations of Cr stress.

### Effects of high concentration of Cr on photosynthesis physiology

The growth of tea has close relations with photosynthesis. Table 3 shows that Tr, Pn, Gs and Ci were negatively correlated with the concentration of Cr, and the correlation coefficient were -0.93\*\*, -0.89\*, -0.86\* and -0.99\*\*. Tr, Pn, Gs and Ci concentrations dependently declined and reached minimum in the Cr concentration of 600 mg·kg<sup>-1</sup> compared with the control group. These results were consistent with the growth trend. This

**Table 1.** Effects of high concentration of Cr stress on tea plant growth.

Treatment	Root	Stubble	Stem	Branch	Leave
Cr0	35.48 ± 2.90 <sup>a</sup>	22.34 ± 1.64 <sup>a</sup>	32.45 ± 1.77 <sup>a</sup>	37.11 ± 3.71 <sup>a</sup>	37.53 ± 3.56 <sup>a</sup>
Cr1	26.61 ± 1.70 <sup>b</sup>	16.60 ± 1.25 <sup>b</sup>	23.22 ± 3.57 <sup>b</sup>	22.23 ± 1.70 <sup>b</sup>	24.90 ± 1.42 <sup>b</sup>
Cr2	22.21 ± 1.18 <sup>b</sup>	13.44 ± 1.53 <sup>bc</sup>	19.96 ± 1.49 <sup>b</sup>	17.42 ± 0.61 <sup>bc</sup>	19.71 ± 1.77 <sup>bc</sup>
Cr3	20.16 ± 3.56 <sup>b</sup>	13.18 ± 0.95 <sup>bc</sup>	15.88 ± 0.45 <sup>bc</sup>	14.91 ± 0.52 <sup>bc</sup>	17.17 ± 2.29 <sup>bc</sup>
Cr4	18.89 ± 0.86 <sup>b</sup>	10.68 ± 1.00 <sup>c</sup>	10.85 ± 1.63 <sup>c</sup>	11.48 ± 1.00 <sup>c</sup>	12.51 ± 1.07 <sup>c</sup>

Within each column, values followed by the same letter are not significantly different at the 5% level.

**Table 2.** Effects of different concentrations of Cr on the content and composition of chlorophyll.

Treatment	Chlorophyll a (mg·g <sup>-1</sup> )	Chlorophyll b (mg·g <sup>-1</sup> )	Chlorophyll a + b (mg·g <sup>-1</sup> )	Chlorophyll a/b (mg·g <sup>-1</sup> )
Cr0	2.79 ± 0.01 <sup>a</sup>	1.16 ± 0.01 <sup>a</sup>	3.96 ± 0.02 <sup>a</sup>	2.41 ± 0.02 <sup>a</sup>
Cr1	2.41 ± 0.14 <sup>b</sup>	1.02 ± 0.05 <sup>ab</sup>	3.24 ± 0.02 <sup>b</sup>	2.34 ± 0.03 <sup>ab</sup>
Cr2	2.04 ± 0.07 <sup>c</sup>	0.96 ± 0.02 <sup>bc</sup>	3.02 ± 0.07 <sup>b</sup>	2.17 ± 0.12 <sup>bc</sup>
Cr3	1.74 ± 0.11 <sup>cd</sup>	0.87 ± 0.02 <sup>c</sup>	2.57 ± 0.11 <sup>c</sup>	2.00 ± 0.07 <sup>cd</sup>
Cr4	1.44 ± 0.09 <sup>d</sup>	0.72 ± 0.08 <sup>d</sup>	2.25 ± 0.11 <sup>d</sup>	1.80 ± 0.05 <sup>d</sup>

Within each column, values followed by the same letter are not significantly different at the 5% level.

**Table 3.** Effects of different Cr concentrations on photosynthesis of tea plant.

Treatment	Tr (μmol·m <sup>-2</sup> ·s <sup>-1</sup> )	Pn (μmol·m <sup>-2</sup> ·s <sup>-1</sup> )	Gs (mol·m <sup>-2</sup> ·s <sup>-1</sup> )	Ci (μL·L <sup>-1</sup> )
Cr0	2.28 ± 0.09 <sup>a</sup>	11.75 ± 0.52 <sup>a</sup>	0.15 ± 0.01 <sup>a</sup>	273.8 ± 5.62 <sup>a</sup>
Cr1	2.12 ± 0.19 <sup>ab</sup>	11.45 ± 0.31 <sup>ab</sup>	0.14 ± 0.01 <sup>ab</sup>	236.33 ± 8.57 <sup>b</sup>
Cr2	2.06 ± 0.17 <sup>ab</sup>	11.02 ± 0.80 <sup>ab</sup>	0.13 ± 0.01 <sup>ab</sup>	231.67 ± 9.33 <sup>b</sup>
Cr3	1.74 ± 0.15 <sup>b</sup>	9.86 ± 0.65 <sup>bc</sup>	0.12 ± 0.01 <sup>bc</sup>	219.50 ± 8.53 <sup>b</sup>
Cr4	1.64 ± 0.18 <sup>b</sup>	9.18 ± 0.30 <sup>c</sup>	0.10 ± 0.01 <sup>c</sup>	215.75 ± 10.62 <sup>b</sup>

Within each column, values followed by the same letter are not significantly different at the 5% level.

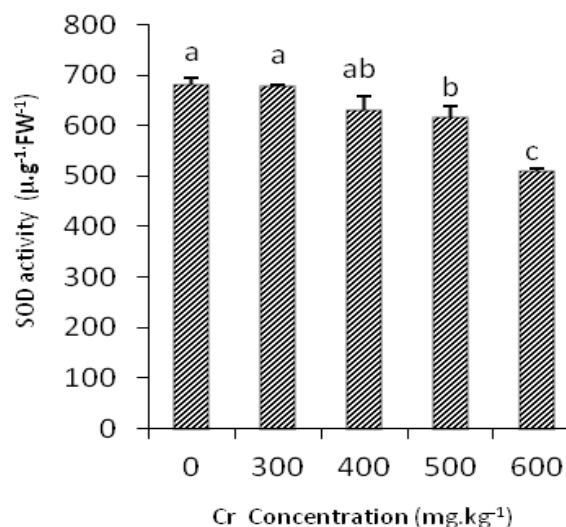
suggest that photosynthesis and growth of tea plant were inhibited by high concentration of Cr.

### Effects of high concentration of Cr on the resistance indicators of tea

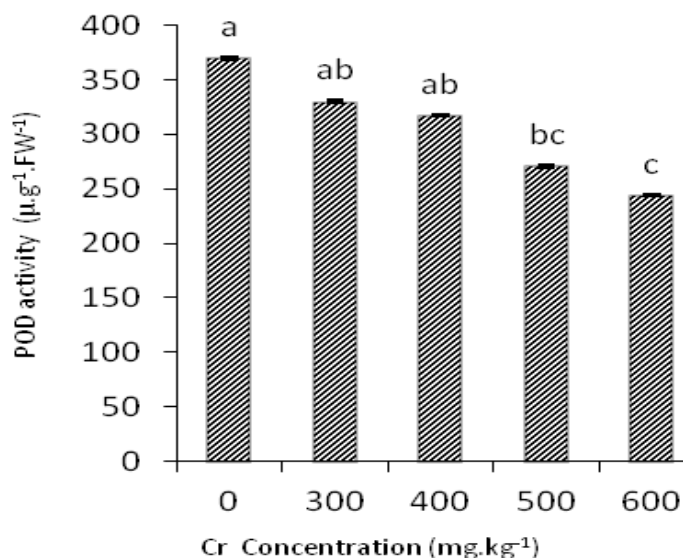
Adverse conditions can affect active oxygen metabolism of plant. The increasing oxygen content in plants can also induce SOD activity increase. When oxygen content is higher than normal level, it can destroy various kinds of cell functions and restrain SOD activity (Ding et al., 2004). POD extensively in plant material and energy metabolism plays a major role in plant respiratory metabolism and physiological resistance. The elevation of POD activity can catalyze H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O and prevent effectively the accumulation of H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub>. It can also reduce the harm caused by free radical to the structure and function of the membrane (Sun et al., 2006; Pauls and Thompson 1984; Vetanovetz and Peterson, 1990). CAT is an important component of plant antioxidant

enzymes system and, it can remove excess H<sub>2</sub>O<sub>2</sub> in plant cell and make H<sub>2</sub>O<sub>2</sub> at normal level in the plant to protect membrane structures (Li et al., 2008).

As shown in Figures 1 to 3, the activities of SOD, POD and CAT in the tea gradually reduced with increasing Cr concentration and showed significantly negative correlation coefficient of -0.81\*, -0.96\*\* and -0.96\*\*, respectively. The activities of SOD, POD and CAT in tea decreased in a Cr concentration-dependent manner. Statistically, Cr4 treatment was significantly lower than for the control (Cr0). This shows that high concentration of Cr can destroy antioxidant enzymes system of tea plant. Moreover, MDA is the final product of lipid peroxidation, and its content can reflect stress tolerance of plants (Liu, 2006; Sugiyama, 1994). From Figure 4, it can be observed that as Cr concentration increased, the MDA content of Pingyang Tezao tea gradually increased and showed a significantly negative correlation and the correlation coefficient was 0.99\*\*. This shows that high concentration of Cr can promote lipid peroxidation; meanwhile, it can also damage the balance of reactive



**Figure 1.** Effects of different concentrations of Cr on leaf SOD activity in tea plant.



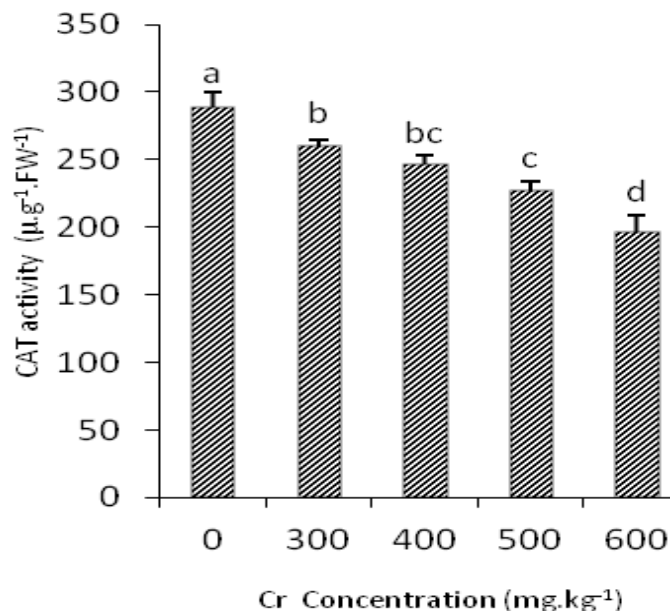
**Figure 2.** Effects of different concentrations of Cr on leaf POD activity in tea plant.

oxygen in tea plant.

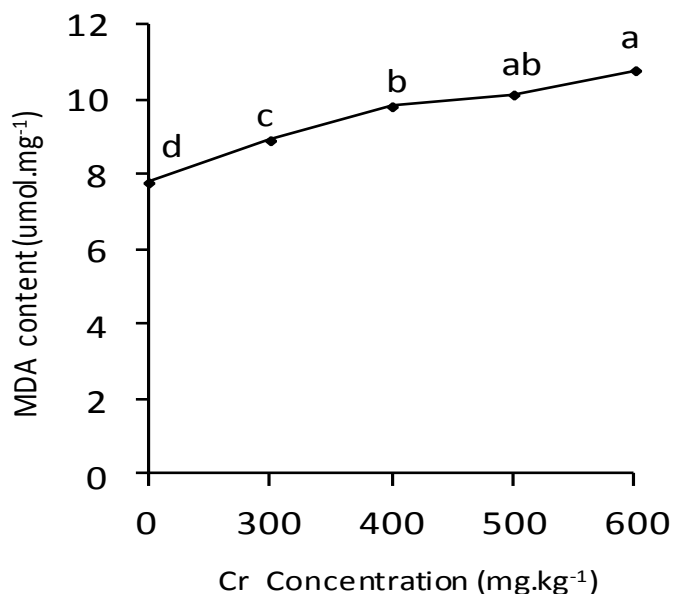
Free Pro is one of the most important osmotic in plant, and plays important physiological roles in osmoregulation, stability of the cell structure and reducing oxidative stress (Li et al., 1999; Metha et al., 1999). Therefore, Pro content is taken as an index which reflects multiple resistance of plant. As indicated in Figure 5, Pro content was significantly positively correlated with Cr concentration, and the correlation coefficient was 0.99\*\*. With Cr concentration increase, Pro content was higher

than for normal subjects by 29.45, 33.02, 43.25 and 45.67%. This shows that the cell membrane structure of leaves was damaged, which promoted the increasing content of proline to regulate osmotic pressure.

Cell membrane has selective permeability and the characteristic is one of physiological indicators that evaluate plant according to reaction modes (Zhao et al., 2002). However, the relative conductivity (RC) of leaves is an index of cell membrane permeability. From Figure 6, we can observe that the RC of Pingyang Tezao tea had



**Figure 3.** Effects of different concentrations of Cr on leaf CAT activity in tea plant.

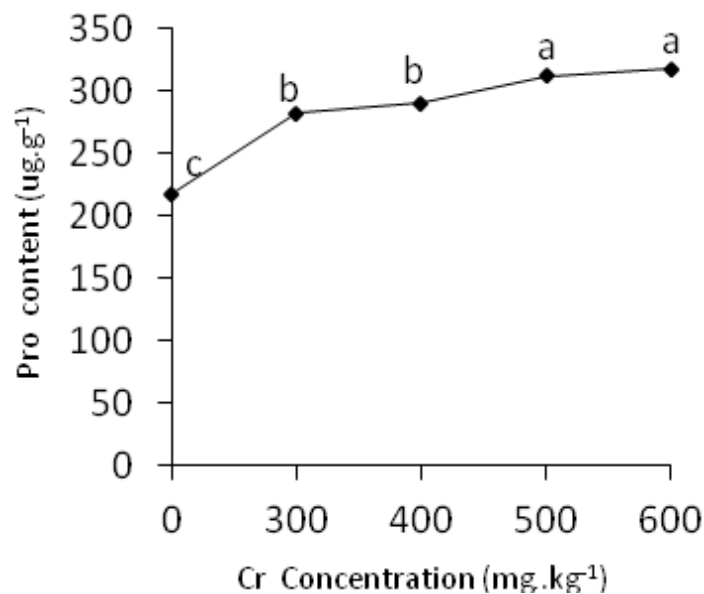


**Figure 4.** Effects of different concentrations of Cr on leaf MDA content in tea plant

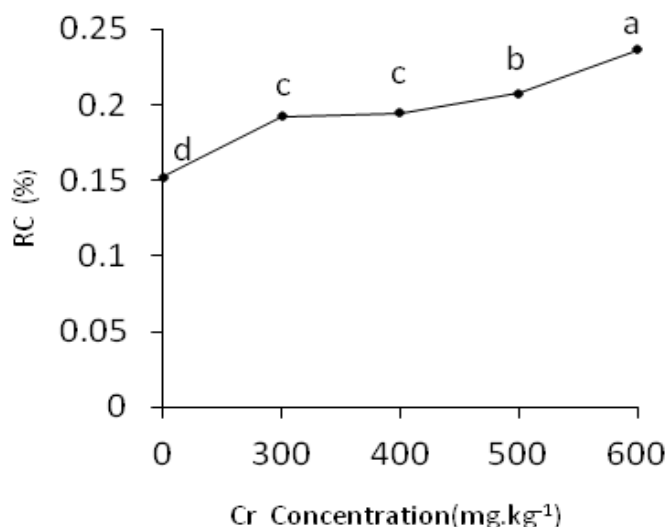
positive correlation with Cr concentration, and the correlation coefficient was 0.97\*\*. The RC increased with Cr concentration increase and reached maximum when Cr concentration was 600 mg.kg<sup>-1</sup>. In comparison with the control group, the RC increased by 26.71, 28.22, 36.58 and 55.46%, which indicates that high concentration of Cr could damage the structure and function of cell membrane.

#### Effects of high concentration of Cr on absorption and accumulation of tea

From Table 4, we can see that the content of Cr in different tea organs significantly increased with Cr concentration increase and reached maximum when Cr concentration was 600 mg.kg<sup>-1</sup>. Cr content in different organs was in the order of: stubble > root > leaf > stem



**Figure 5.** Effects of different concentrations of Cr on leaf Pro content in tea plant.



**Figure 6.** Effects of different concentrations of Cr on leaf CMP in tea plant.

> branch  $\approx$  new stem. The accumulation in stubble was 5~17 times that of the root, 49~149 times of branch, 35~90 times of stem, 11~31 times of leaf, and 59~126 times of new stem. Meanwhile, the content of Cr was a positive correlation with Cr concentration (Table 5).

## DISCUSSION

Tea plants have different symptoms in different concentrations of Cr and different time. In our study, tea

showed obvious symptoms during later stages of processing, and the bud and growth of tea were inhibited, while the leaves became brown and yellow and the damage increased with Cr concentration increases. Those symptoms led to a decline in tea growth and production. Tang et al. (2008) provided similar results in a solution culture experiment. The yellowing leaves were probably caused by the interaction between the Cr and iron overload of leaves. The interaction reduced the absorption of iron and the synthesis of chlorophyll in tea plant, thus showing that high concentrations of Cr could

**Table 4.** The accumulating amounts of Cr in different parts of tea plants ( $\text{mg}\cdot\text{kg}^{-1}$ ).

Treatment	The Cr contents in different parts of tea plant					
	Stubble	Root	Stem	Branch	Leaf	New stem
Cr0	$5.26 \pm 0.14^e$	$1.44 \pm 0.12^e$	$0.62 \pm 0.04^c$	$1.24 \pm 0.04^c$	$1.22 \pm 0.024^e$	$0.62 \pm 0.06^d$
Cr1	$156.7 \pm 5.48^d$	$29.61 \pm 1.03^d$	$5.53 \pm 0.27^b$	$3.36 \pm 0.22^b$	$16.33 \pm 0.19^d$	$3.95 \pm 0.08^c$
Cr2	$197.48 \pm 1.53^c$	$47.78 \pm 0.95^c$	$5.50 \pm 0.43^b$	$4.27 \pm 0.10^b$	$19.92 \pm 0.02^c$	$4.74 \pm 0.12^{bc}$
Cr3	$269.10 \pm 6.10^b$	$57.33 \pm 0.20^b$	$5.80 \pm 0.23^b$	$4.45 \pm 0.42^b$	$24.50 \pm 0.35^b$	$5.30 \pm 0.04^b$
Cr4	$499.40 \pm 7.68^a$	$94.89 \pm 1.17^a$	$14.22 \pm 0.67^a$	$10.29 \pm 0.48^a$	$44.31 \pm 0.25^a$	$8.52 \pm 0.45^a$

Within each column, values followed by the same letter are not significantly different at the 5% level.

**Table 5.** The correlations between the Cr content in different parts of tea plants (y) and the Cr concentrations (x).

Organ	Correlation	Correlation coefficient
Stubble	$y = 0.727x - 36.148$	0.8558
Root	$y = 0.1435x - 5.4398$	0.9137
Stem	$y = 0.0183x - 0.2713$	0.7399
Branch	$y = 0.0123x + 0.2808$	0.7129
Leave	$y = 0.0634x - 1.5704$	0.8791
New stem	$y = 0.0119x + 0.3532$	0.9293

cause damage and inhibit the growth of tea, as well as disrupt its normal physiology activity. Chlorophyll is the main pigment of plant photosynthesis. Its content reflects the activity of plant photosynthesis. This study indicates that the content of chlorophyll a, chlorophyll b and total chlorophyll decreased with increase in Cr concentration. This result was consistent with Wang et al. (2008) and Shi (2004). The possible mechanism underlying the decrease of chlorophyll was that high concentration of Cr changed the normal structure of catalytic enzymes of chlorophyll production, and also inhibited enzyme activity and chlorophyll synthesis. Also, it may be caused by heavy metal and the damage of intracellular membranes (Shi et al., 2001).

The Pn, Tr, Gs and Ci also decreased with the increase of Cr, and high concentration of Cr damaged tea plant and inhibited photosynthesis which was in agreement with the results of Li (2008). This may be as a result that lots of Cr ion combined with nucleic acids, proteins and enzymes in tea plant replaced necessary and particular element and caused their denaturation or inactivation, impeded respiratory metabolism, photosynthesis and cell division (Zhang, 1997; Shi et al., 2003); or it may be that, heavy metals could lead to the decrease of Gs and Pn (Yao et al., 2005).

Adversity stresses can cause metabolism imbalance. SOD can scavenge the free radicals ( $\text{O}_2^{\cdot}$ ) and POD and CAT can clear reactive oxygen radicals ( $\text{H}_2\text{O}_2$ ). These enzymes could maintain the stability of cell membranes and protect plant cell from reactive oxygen (Luo et al., 2004). Our research showed that the activities of SOD, POD and CAT decreased with the increase of Cr con-

centration, and it is possible that the resistance exceeded the threshold value of defense response in high concentration of Cr, which made the activities of protective enzymes decrease and the capacity of scavenging reactive oxygen of protective system weaken gradually. In other words, reactive oxygen metabolism was disrupted and resulted in the increasing content of Pr, MAD and membrane permeability. All these can eventually poison tea plant (Chen et al., 2009). Our data is compatible with these results of the soil culture experiment from Li (2008).

Heavy metal in different parts of tea plant comes directly from the absorption of roots in soil. In this study, we observed that the levels of heavy metal in various parts increased with the increased concentration of Cr, and the increase of heavy metal content in the root was higher than that in other parts. In addition, the accumulating ability of the root of Cr was also the strongest of all organs. Our study regarding the interaction between heavy metal level and Cr content are consistent with previous researches of Tang et al. (2008) and Li (2008) whereas, some other related reports indicated that Cr mainly accumulated in tea root, thereby making it difficult to be absorbed and translocated, and only small amounts of Cr is found aboveground parts (Wang, 2005; Li, 2004; Sun, 2001; Guo, 2005). These results demonstrate that tea root has a strong stranded role of Cr in high concentration of Cr.

In conclusion, our data demonstrate that Cr has different poisonous effect on tea plant with the increasing Cr concentration. The minimum Cr concentration ( $300 \text{ mg}\cdot\text{kg}^{-1}$ ) had poisonous effect on tea plant growth, but the concentration of Cr in this experiment did not lead to tea

plant death. These results demonstrate that the tea plant has good tolerance of Cr.

## ACKNOWLEDGEMENTS

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