# Effects of Three Soil Amendments on Plant Physiological Responses in Cr (III)-contaminated Soil

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Abstract: To evaluate the effect of three soil amendments, i.e., chicken manure (CM), peat (PE) and vermiculite (VE) on plant physiological responses, we planted Lolium perenne (L. perenne) and Pharbitis purpurea (P. purpurea) in soils contaminated with 1000 mg·kg-1 Cr (III) in the laboratory. The results showed that all three amendments promoted plant growth and physiological status. Under Cr (III) stress, compared with the control, the biomass and root length of L. perenne in soil amended with CM were 1.26 and 1.57 times higher, and in P. purpurea, the values were 1.47 and 2.06 times higher, respectively. Principal component analysis (PCA) showed that CM was the best amendment in Cr (III)-contaminated soil. Therefore, CM have the potential to serve as efficient soil amendment for Cr (III)-contaminated soil.

## 1 INTRODUCTION

Chromium (Cr) is regarded as a crucial environmental pollutant due to its many sources and global effect. Cr(III) is the most stable and is the predominant states in the natural environment (Ashraf 2017). Therefore, studying Cr(III)-contaminated soil is of significance.

The application of soil amendments has become an effective method for in situ remediation of Cr-contaminated soil (Huang 2018, Li 2016). Natural soil amendments are promising research materials because of their low cost and natural abundance (Abd 2015, Habashy 2011). Multiple studies have evaluated the application of abundant amendments such as lime, red mud, and compost for remediating soils contaminated with heavy metals(Reijonen 2016, eesley 2010). Extracting Cr from the soil via roots and translocating plants is complex. Different types of plants have differing bioaccumulation capacities with respect to Cr.

In general, we use hyperaccumulators of Cr to remediate the soil and improve the ecological environment of polluted areas. Because the current varieties of Cr-hyperaccumulators are in a relatively short supply, the *L. perenne* and *P. purpurea* herbs that are commonly found in the study area and have a certain tolerance to adverse environmental conditions are selected in this study. *L. perenne* is a perennial herb that undergoes rapid growth and is widely cultivated around the world. *L. perenne* has a well-developed root system, high biomass, and strong tolerance to heavy metals (Bidar 2007). *P. purpurea* is an annual tendril winding herb that can grow in a wide array of soil types. It has a well-developed root system and a strong resistance to drought and other adverse growing conditions (Uva 1997). In addition to remediating contaminated soil, natural amendments can also have a direct or indirect effect on plant growth (Khan 2018). Therefore, the physiological changes during plant growth can also be used to measure the effect of soil conditioning.

Several studies have been conducted to assess the role of amendments in the remediation of metal-contaminated soil. However, no such comprehensive study has been conducted to evaluate the comparative effects of organic and inorganic amendments on plant physiological responses. The aims of this study is to clarify the different functions of the three amendments on the soil-plant systems under Cr (III) stress.



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# 2 MATERIALS AND METHODS

## 2.1 Soil and Amendments

Soil samples were collected from the 0-20 cm interval of a chromium salt factory upstream of the Loushan River in the Licang District of Qingdao, China (36.21°N, 120.39°E). The three soil amendments examined in this study included chicken manure (CM), peat (PE) and vermiculite (VE). Each sample was completely mixed; then, samples and amendments were air dried and passed through a 2-mm sieve.

Some chemical properties of the soil and amendments were estimated according to standard procedures(Nelson 1996). The soil pH: 7.32, organic matter content: 10.47 $g\cdot kg^{-1}$, CEC: 16.48 $cmol\cdot kg^{-1}$, And the basic chemical properties of amendments are presented in Table 1.

Table 1. The basic chemical properties of the three amendments.

| Amendments | pH value | Organic matter content /$g\cdot kg^{-1}$ | total nitrogen /% | Cr(T) /$mg\cdot kg^{-1}$ |
|---|---|---|---|---|
| CM | 7.87 | 45.3 | 20.4 | - |
| PE | 6.10 | 62.4 | 15.8 | - |
| VE | 7.40 | - | - | - |

-: indicates not detected.

## 2.2 Pot Experiment

The experiments in this study were conducted by potting *Lolium perenne (L. perenne)* and *Pharbitis purpurea (P. purpurea)* with organic amendments (CM and PE) and an inorganic amendment (VE) in Cr(III)-contaminated soils. *L. perenne* and *P. purpurea* are more tolerant to Cr when exposed to Cr(III) levels no greater than 1000 $mg\cdot kg^{-1}$(Chen 2017). Therefore, the concentrations of Cr(III) in the soil samples were set at 1000 $mg\cdot kg^{-1}$ in this study. Cr was added uniformly in the form of a $CrCl_3\cdot 6H_2O$ solution and then equilibrated in the laboratory at 25 °C for one week.

Three amendments were added to the soil at a 20 $mg\cdot kg^{-1}$ application concentration and thoroughly mixed. Plastic pots with a diameter of 12 cm and a height of 14 cm were filled with 1 kg of the soil (dry weight, dw). Each amendment and a control (without amendment) were prepared in triplicate. Two sets (one for *L. perenne* and another for *P. purpurea*) of the selected treatments were provided.

Seeds of two plants were obtained from Shangpin Landscaping Engineering Company, Hefei. The seeds were washed with $H_2O_2$ and then with deionized water. Then, the seeds were sown in the selected pots. The pots were kept in a greenhouse with a controlled environment. After germination, there were equal numbers of uniform and healthy seedlings in each pot. All pots were adjusted daily to a water content of 75% and a field capacity (FC) of 100% by weight.

## 2.3 Plant Analysis

The plants were harvested after 30 days of growth. Three healthy and uniform plants were selected to measure the shoot length and root length with a ruler. The soil particles of the roots were removed with tap water and then deionized water, and the shoots of the plants were rinsed with deionized water.

After rinsing, fresh plant samples were used to determine the plant growth and physiological indexes. Other plant roots and shoots were dried in an oven at 70°C separately to a constant weight and weighed to determine the dry matter yield (Kingsbury 1984).

The water content (W) of the plants was calculated by equation (1):

$$W = (FW - DW)/FW \times 100\% \quad (1)$$

where FW is the fresh weight (g) and DW is the dry weight (g).

The root activity was monitored by the triphenyl tetrazolium chloride (TTC) method(Li 2000). The proline content was measured according to Bates et al(Bates 1973). The lipid peroxidation of the plant tissue was measured in terms of the MDA content per the method given by Heath and Packer(Heath 1968).

Fresh plant samples were used to determine the activities of antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). The SOD activity was determined by the method of Beauchamp and Fridovich(Beauchamp 1971); the POD activity was measured using the method of Batish et al (Batish 2006); and the CAT activity was determined by Cakmak and Marschner (Cakmak 1992).

## 2.4 Statistical Analysis

The Statistical Product and Service Solutions software package (SPSS, version 20.0) was used in the statistical analysis. One-way analysis of variance (ANOVA) using Duncan's multiple range test (P =

0.05) was conducted to determine the statistical significance of the differences among samples. The multivariate statistical technique of principal component analysis (PCA) was used to investigate and identify the important components explaining most of the variances in the data.

# 3 RESULTS AND DISCUSSION

## 3.1 Plant Growth and Biomass

The effects of CM, PE and VE on the growth indexes of the two plants under Cr stress are shown in Table 2. The rank order of the three amendments in terms of the positive effect on plant growth under Cr (III) stress was CM > PE ≥ CK ≥ VE, and the effect was better on *P. purpurea* than on *L. perenne*. The biomass of *L. perenne* and *P. purpurea* in the CM group exceeded 26.1% and 46.7% of that of CK, and the shoot length of these plants reached 1.28 and 1.71 times higher than that of CK, respectively. The two organic amendments caused the root length of *L. perenne* to reach 1.57 and 1.16 times higher than that of CK in the Cr (III) treatments, and that of *P. Purpurea* reached 2.06 and 1.36 times higher than that of CK, respectively. The PE group had a significant increase in the root length of the two plants ($P < 0.05$), which indicated that the plant tolerance and resistance were increased. There was no significant difference in the plant growth indexes of the VE and CK groups.

Table 2. Effects of amendments on the growth of two plants under Cr (III) stress.

| Amen-dments | *L. perenne* | | |
|---|---|---|---|
| | shoot length/cm | root length /cm | biomass/mg DW·plant$^{-1}$ |
| CK | $21.37\pm0.68^{b}$ | $7.90\pm0.44^{c}$ | $10.01\pm0.13^{b}$ |
| CM | $27.27\pm1.16^{a}$ | $12.40\pm0.62^{a}$ | $12.62\pm0.45^{a}$ |
| PE | $20.07\pm1.04^{b}$ | $9.17\pm0.35^{b}$ | $9.78\pm0.52^{b}$ |
| VE | $19.77\pm0.42^{b}$ | $7.30\pm0.20^{c}$ | $8.80\pm0.32^{bc}$ |
| Amen-dments | *P. purpurea* | | |
| | shoot length/cm | root length /cm | biomass/mg DW·plant$^{-1}$ |
| CK | $10.61\pm0.42^{bc}$ | $7.33\pm0.30^{c}$ | $29.63\pm0.97^{c}$ |
| CM | $18.13\pm0.37^{a}$ | $15.12\pm0.20^{a}$ | $43.17\pm1.33^{a}$ |
| PE | $10.90\pm0.48^{b}$ | $9.94\pm0.54^{b}$ | $32.62\pm0.35^{b}$ |
| VE | $9.68\pm0.47^{c}$ | $6.26\pm0.22^{d}$ | $27.33\pm0.55^{c}$ |

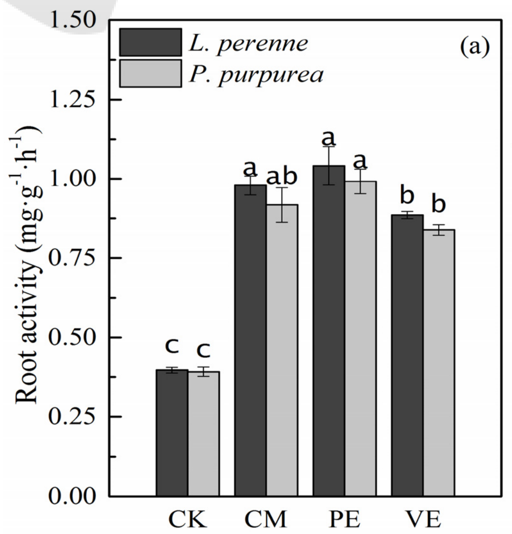
Different letters (a, b, c) indicate significant differences of index values between the different treatments.

## 3.2 Root Activity, Proline and MDA

The physiological and metabolic effects of the three amendments on Cr (III) are shown in Figure 1. Under Cr (III) stress, the three amendments significantly ($P < 0.05$) promoted the root activity of the plants, according to Figure 1a. PE had the greatest influence on the root activity of *L. perenne* and *P. purpurea*, which was 2.6 and 2.5 times higher than that of the control plants, respectively. VE had the minimum effect, but it was still more than 123.7% and 112.4% of that of CK, respectively.

Proline (Pro) is an osmoregulatory substance in plants (Vernay 2008). Both CM and PE significantly decreased the Pro content ($P < 0.05$) of the two plants, and VE had the least effect under Cr(III) stress (Figure 1b). Compared with the control, CM and PE decreased the Pro content to 66.8% and 60.9% in *L. Perenne*, 62.5% and 61.6% in *P. Purpurea*, respectively. Thus, PE can alleviate the osmotic stress of Cr(III) in plants to a greater extent than CM.

The content of MDA reflects the degree of cell membrane lipid peroxidation in plants and reflects the reactive oxygen species (ROS) content in plants(Vernay 2008). Under Cr(III) stress, CM and PE decreased the content of MDA in the two plants. VE had no significant ($P < 0.05$) effect (Figure 1c). CM reduced the MDA content in *L. perenne* and *P. purpurea* to 74.2% and 74.5% of that of CK, respectively.



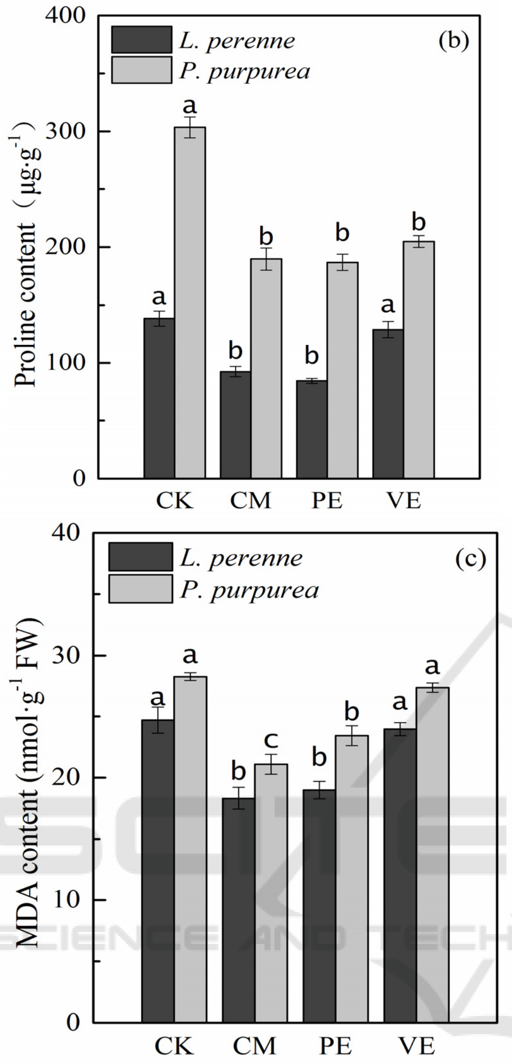



Figure 1: Effects of amendments on the physiological indexes of two plants under Cr(III) stress.

### 3.3 Cr Uptake and Transport in Plants

The effects of the three amendments on the activity of antioxidant enzymes in plants under Cr(III) stress are shown in Figure 2. The three amendments enhanced the antioxidant enzyme activities of the plants(Islam 2015). Under Cr(III) stress, the positive effect of CM and PE on the antioxidant enzyme activities in plants did not show a significant difference ($P < 0.05$), and VE had less of an effect. In CM group, the SOD activities of *L. perenne* and *P. purpurea* were 24.0% and 99.5% higher than that of CK (Figure 2a). The POD activity was 78.6% and 40.1% higher than that in the CK group (Figure 2b). The CAT activity, which was the most sensitive parameter to the amendments, was 44.4% and 47.3% higher than that in the CK group, respectively (Figure 2c).

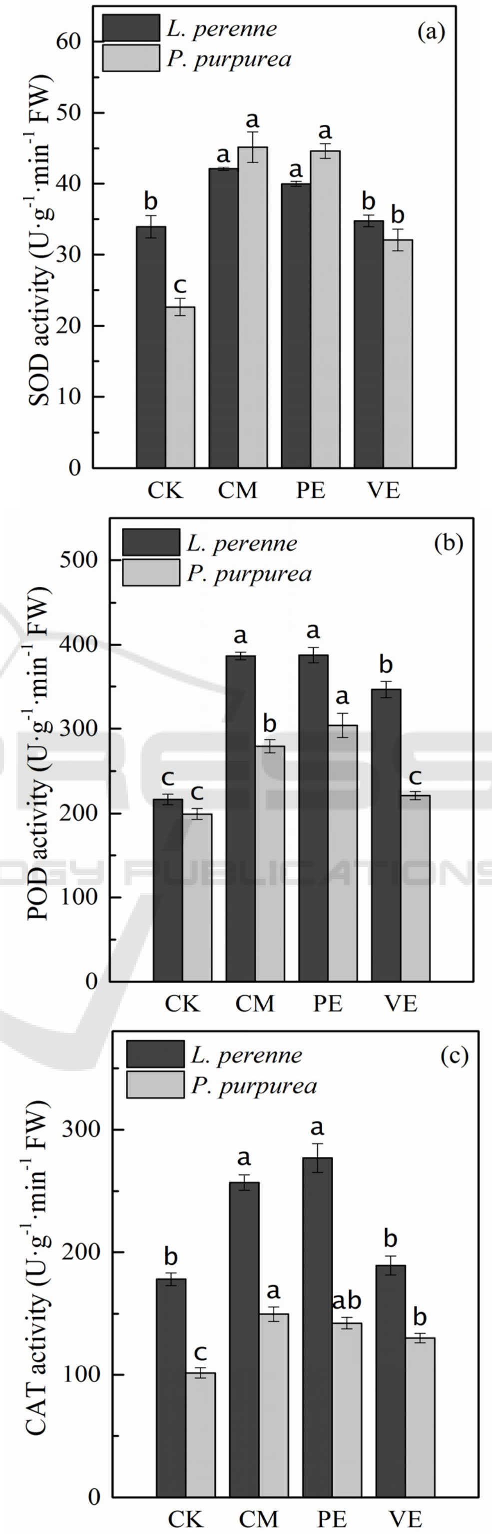
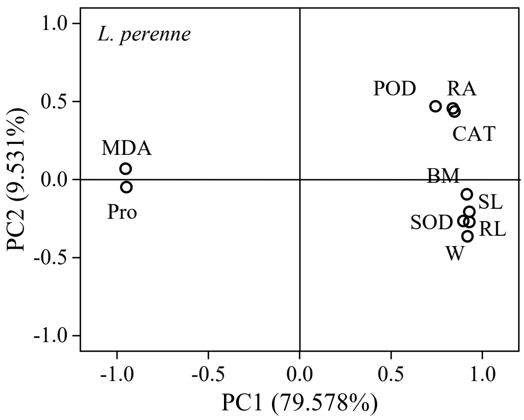



Figure 2: Effects of amendments on the antioxidant enzyme activities of two plants under Cr(III) stress.

### 3.4 Principal Components Analysis

There are many variables involved in this complex experimental system, and the rank order of the amendments in terms of their plant growth and physiological indexes is different (Table 3, Figure 1 and Figure 2). PCA was implemented to determine the components in the large data set. The growth, physiological and biochemical indexes of two plants were included as the analysis variables. The results are shown in Figure 3.

From PCA, principal component 1 (PC1) and PC2 explained 79.578% and 9.531% of the total variance in the *L. perenne* groups, accounting for 89.109% of the total variance (Figure 3a) and indicating that most information associated with the growth and physiological indexes of the plants was involved in the first two PCs. PC1 displayed remarkable positive correlations with the root length (0.929), shoot length (0.929), biomass (0.915) and moisture content (0.919), and the contents of MDA and Pro exhibited prominent negative correlations with PC1 (-0.953 and -0.948). However, no parameter was correlated with PC2. In the *P. purpurea* groups, PC1 and PC2 explained 69.530% and 18.598% of the total variance in the soils, accounting for 88.128% of the total variance (Figure 3b). PC1 exhibited distinct positive correlations with the root length (0.934), SOD activity (0.943), shoot length (0.920) and root activity (0.892), and the MDA content displayed a negative correlation with PC1 (-0.873). However, PC2 had a moderate positive correlation with only the CAT activity.



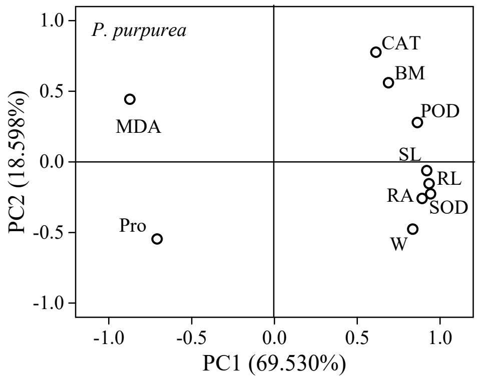




Figure 3: PCA of growth and physiological parameters of two plants (SL=shoot length, RL=Root length, BM=Biomass, W=Water content, RA=Root activity, MDA=Malondialdehyde content, Pro=Proline content, SOD=Superoxide dismutase activity, POD=Peroxidase activity, CAT=Catalase activity).

The above data analysis shows that after application of amendments, the growth and physiological states of the plants were improved. According to the data score of the response indicators of the two plants in Cr(III)-contaminated soil, we can conclude that the rank order of the three amendments in terms of the comprehensive improvement was CM > PE > VE. The results provide guidelines for selecting amendments and plants for the remediation of Cr(III)-contaminated soil.

Applying the comprehensive analysis of the plant growth physiological indexes and the Cr bioavailability, we found that the effects of the three amendments were different. The two organic amendments (CM and PE) were much more effective than the inorganic amendment (VE) at improving the function of the soil-plant system due to the rich organic matter content (Choppala 2015). CM reduced the stress of Cr to the plants by reducing the bioavailability of Cr in the soil. PE enhanced the accumulation of Cr by the plants by changing the physical and chemical properties of the soil and promoting plant root growth. Thus, PE can enhance plant Cr tolerance. The improvement with the VE amendment was due to the large amount of silicon (aluminum) hydroxyl groups and metal hydroxide groups, which effectively adsorb heavy metals by ion exchange (Bradl 2004).

# 4 CONCLUSION

In this study, we found that all three amendments have a positive effect on plant growth in Cr(III)-contaminated soil. The rank order of the three amendments in promoting plant growth was CM > PE > VE under Cr(III) stress. These results may be useful for screening effective amendments and plant species for the remediation of Cr(III)-contaminated soil.

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