EFFECTS OF Pb AND Cd STRESS ON THE PHOTOSYNTHETIC PHYSIOLOGICAL CHARACTERS OF POTATO IN HEAVY METAL POLLUTION OF SOIL

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Abstract. Study on the effects of Pb and Cd stress on the photosynthetic physiological characters of potato. The single-factor randomized block experiments with the potato variety Xishu 1 as the test material. The photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), intercellular CO_2 concentration (Ci) and SPAD value of the potato decreased with the increased concentrations and prolonged treatment. As the concentrations of Pb and Cd continued to increase, most of the photosynthetic physiological indices tended to be stable. But in the treatment with high concentration of Cd, the SPAD value decreases sharply. The potato photosynthetic system has certain tolerance to Pb and Cd, and the effect of Cd on the photosynthesis of potato is greater than that of Pb.
Keywords: *Pb and Cd, potato, photosynthetic, physiological characters, stress*

Introduction

With the progress of the human society and the accelerated industrialization process, environmental pollution has become one of the most prominent ecological problems in the world, including the heavy metal pollution of soil (Wan et al., 2008). Heavy metal pollution has been one of the hotspots in ecological and environmental biology research. So far, there have been a lot of studies on the effects of heavy metals on the biological morphology, their physiological and biochemical effects and the resistance mechanism of plants against heavy metal pollution (Kong et al., 2007; Zhou et al., 2007; Li et al., 2008). The current research mainly focuses on the toxic effects of heavy metals on plants, toxic mechanisms and the resistance mechanisms of plants, but little research involves the photosynthetic physiological characters of plants under heavy metal stress.

At present, 20 million hm^2 of soil has been polluted by heavy metals such as Pb and Cd in China, accounting for about one-fifth of the total cultivated land area (Lee, 2016). Every year, the economic loss caused by heavy metal pollution can be as high as 20 billion RMB (Gu et al., 2003). According to statistics, the land area suffering from heavy metal pollution accounts for 64.8% of the total wastewater irrigation area, including 46.7% mild pollution, 9.7% moderate pollution and 8.4% serious pollution (Chen et al., 2002). At present, the soil in the suburbs of most cities in China has been polluted to varying degrees. In many places, the contents of heavy metals such as Pb and Cd in grains, vegetables, fruits and other foods already exceed the standards and are close to the critical values. According to the survey conducted by the Ministry of Agriculture, among the 320 key polluted areas including suburbs, wastewater irrigation areas and industrial mines in 24 provinces (cities), 606,000 hm^2 of field crops suffer from excessive pollution, accounting for 20% of the total area monitored. In particular, the output and area of agricultural products with excessive heavy metal content account for about 80% of

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the total output and area of the agricultural products with excessive amount of pollutants. The pollution of Pb, Cd, Hg, Cu and their combinations is the most serious (Hou et al., 2017). Pb and Cd are the elements toxic to the plant growth. A large number of studies have confirmed that Pb can affect the antioxidant enzyme systems of plants (Sun et al., 2009; Cai et al., 2012); that the root tips of Cd-polluted broad bean seedlings turn dark brown and suffer from necrosis (Mo and Li, 1992); and that Cd can inhibit the growth of corn, wheat (Parlak, 2016), cucumber (Chen, 1990) and tomato (Moral et al., 1994), etc., and result in symptoms like leaf chlorosis and yellowing, which all affect the crop yield. In recent years, through research, Wang et al. (2009) found that the chlorophyll content, photosynthetic rate, transpiration rate and other indicators of radish leaves significantly dropped with the increase in the concentration of the Pb and Cd mixed solution (Wang and Shi, 2008); Wang et al. (2009) found that low-concentration Pb and Cd mixed solution has certain promoting effects on various physiological indicators of wheat, and that with the increase of concentration, the synthesis of chlorophyll will be blocked (Wang and Zheng, 2009). This reveals that Pb and Cd have certain impacts on the photosynthesis of plants; however, no research has been reported on the effects of Pb and Cd on the photosynthetic physiological characters of potato.

Potato (*Solanum tuberosum* L.), an annual herb of the Solanaceae family, is an important type of staple food and vegetable (Furrer et al., 2018). Potato is rich in nutrients and has high edible value. The starch in the dry matters' accounts for 75%-80%, which can be easily absorbed by the human body; the protein availability is 71%, which is 21% higher than that of grain, and what is more, it is rich in vitamin C (Li, 2013). In China, potato is an important kind of export goods; and in agricultural production, it is an excellent fore crop for cereal crops, and also an excellent crop for intercropping and replanting. Therefore, developing potato production is of great significance to improving the living standards of the people and promoting the development of light industry, food industry and grain production. With the development of industrialization and urbanization, large amount of Pb and Cd are continuously entering the soil, resulting in more and more serious pollution, which brings great harm to humans, environment and agriculture. So far, heavy metal elements have been accumulated to varying degrees in the soil (Bat, 1997; Fang, 2015; Jing et al., 2018; Pozza and Bishop, 2019), especially in suburban soil. The contents of Pb and Cd in soil have already greatly exceeded the world soil background values. For plant growth, Pb and Cd are non-essential elements, and both of them can adversely affect the chlorophyll synthesis and antioxidant enzymes of plants. When the dose exceeds a certain level, they may seriously affect the physiological metabolism of plants, hinder their growth and even lead to plant deaths (Bai et al., 2012). However, no research has been reported on the effects of Pb and Cd stress on the photosynthetic physiological characters of potato. This experiment explored the relationship between the photosynthetic physiological characters of potato and the heavy metal pollution of soil, so as to reveal the response of the potato photosynthetic system to heavy metal stress, and provide theoretical guidance for potato production.

Materials and Methods

Test materials

The potato variety tested was Xishu No. 1, supplied by Plateau and Subtropical Crop Laboratory, Xichang University.

The height, diameter, volume of pots is 150 g, 35 cm, 1750 cm^3.

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Experimental design

The experiment was carried out in the phytotron at Xichang University. We arrange the condition of enviroment on normal status which potato can grow healthy. During the experiment, the indoor CO_2 concentration was (450±50) μmol·mol^{-1}, the light intensity (420±50) μmol·m^{-2}·s^{-1}, the relative humidity (55±8)%, the daytime temperature (25±2)ºC, the nighttime temperature (15±2)ºC and the illumination 12 h per day. The test soil was collected from the surface soil in the experiment field at Xichang College. The physical and chemical properties were as follows: the pH value was 6.30 (water)/7.71 ($CaCl_2$), the total P content was 441 mg·kg^{-1}, the total N content 853 mg·kg^{-1}, the total K content 2313 mg·kg^{-1}, CEC 11.23 meq/100g, the organic matter content 25.1 mg·kg^{-1}, and the contents of the heavy metals Pb and Cd 20 and 0.2 mg·kg^{-1}, respectively. The Pb stress treatment levels were respectively 0(CK), 200, 500 and 1000 mg·kg^{-1}, and Pb was added in the form of $Pb(CH_3COO)_2$; the Cd stress treatment levels were 0(CK), 20, 50, 100 mg·kg^{-1}, and Cd was added in the form of $CdCl_2 \cdot 2.5H_2O$. After being treated, the soil was mixed well and stabilized for two weeks, and then potato seedlings were transplanted. The plants with roughly the same weight and height (about 8-10 cm) were selected and randomly allocated to the soil with different concentrations. For each treatment level, 16 pots were planted, and each pot had one plant. After transplantation, the water was added by the weighing method to maintain the soil moisture at about 60% of the field moisture capacity. After 30 days of heavy metal stress treatment, the photosynthetic physiological indices were determined.

Determination of the photosynthetic physiological indices

The potato leaves (the 3rd and 4th pairs of leaves of the main stem from top down) were measured during the period from 10:00 to 11:30 using the CI-340 portable photosynthetic measure system (CID (Beijing) Ecological Scientific Instrument Co., Ltd.). The indices determined included net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs) and intercellular CO_2 concentration (Ci). At the same time, the SPAD value of the leaves was measured by SPAD-502 (Minota, Japan) chlorophyll meter. For each leaf, the indicators were determined for three times.

Data analysis

The above experiment was repeated 3 times, and the average values were taken as the results. And then statistical analysis and plotting were performed using the software SAS 8.2 and Excel.

Results

Effects of Pb and Cd on the photosynthetic physiological characters of potato

Effects of Pb on the photosynthetic physiological characters of potato

It can be seen from *Fig. 1* that under different concentrations of Pb, the photosynthetic physiological characters of potato showed different variation patterns. Under the action of Pb, the photosynthesis rate of potato decreased with the increase of the Pb concentration, and the treatment groups were significantly different from the control group. When the concentration of Pb was 200 mg·kg^{-1}, the net photosynthetic rate decreased the most – by 22.97%, and then the decline gradually slowed down.

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When the concentration of Pb was 1000 mg·kg^{-1}, the net photosynthetic rate was 52.55% of that in the control group. After Pb treatment, Tr of the potato leaves decreased with the increase of the Pb concentration, and the results of the treatment groups were significantly different from those of the control group. When the concentration of Pb exceeded 200 mg·kg^{-1}, the decrease of Tr became slower; and when the concentration of Pb was 1000 mg·kg^{-1}, Tr was 74.66% of that in the control group. When the concentration of Pb was 200 mg·kg^{-1}, Gs was not significantly different from that in the control group. With the increase of the Pb concentration, the decrease of Gs became faster. When the concentration of Pb was 1000 mg·kg^{-1}, Gs was 74.67% of that in the control group. With the increase of the Pb concentration, the decrease of Ci gradually slowed down and the content tended to be stable. At the Pb concentration of 500 and 1000 mg·kg^{-1}, Ci was 84.21% and 83.32% of that in the control group, respectively.

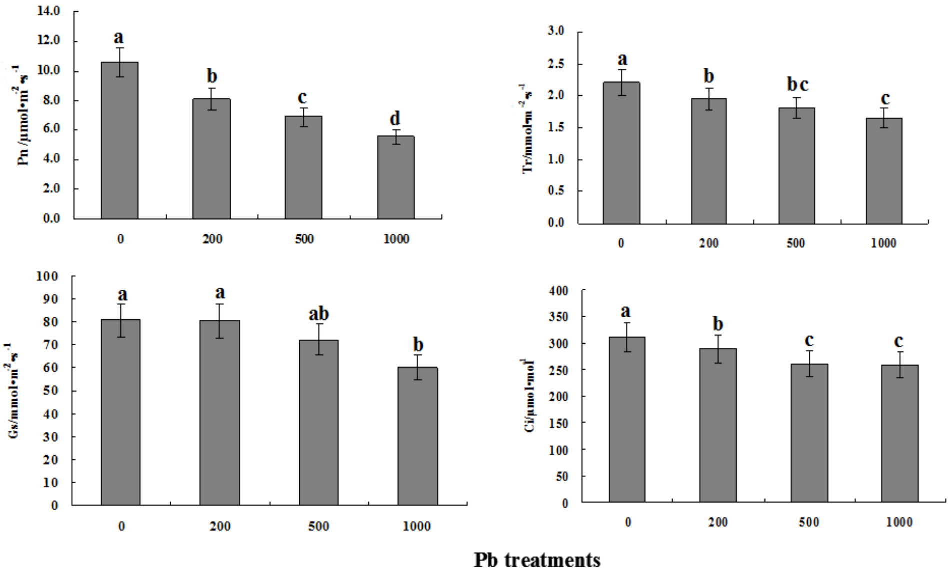

Figure 1. *Effects of Pb on the photosynthetic physiological indices of potato. The data are displayed as mean±SD, n=3. Different letters indicate the significant differences between treatments, P<0.05*

Effects of Cd on the photosynthetic physiological characters of potato

It can be seen from *Fig. 2* that under different concentrations of Cd, the photosynthetic physiological characters of potato showed different variation patterns. Under the action of Cd, the photosynthesis rate of potato decreased with the increase of the Cd concentration. When the concentration of Cd was 20 mg·kg^{-1}, the photosynthetic rate of potato did not differ much from that in the control group; but when the concentration was 50 mg·kg^{-1}, the net photosynthetic rate decreased the most – by 26.75%, and after that, the decline gradually slowed down. When the concentration of Cd was 100 mg·kg^{-1}, the net photosynthetic rate was 50.28% of that in the control group. After Cd treatment, Gs showed a different decline trend from that in the Pb treatment – it decreased more slowly with the increase of the Cd concentration. When the concentration of Cd exceeded 50 mg·kg^{-1}, Gs tended to be stable; and when the concentration of Cd was 1000 mg·kg^{-1}, Gs was 68.04% of that in the control group.

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With the increase of the Cd concentration, Ci first declined and then increased. When the Cd concentration was 50 mg·kg^{-1}, Ci declined the most and was 89.39% of that in the control group. When the Cd concentration was 50 mg·kg^{-1}, Ci started to increase. At the Cd concentration of 100 mg·kg^{-1}, Ci increased to 98.47% of that in the control group.

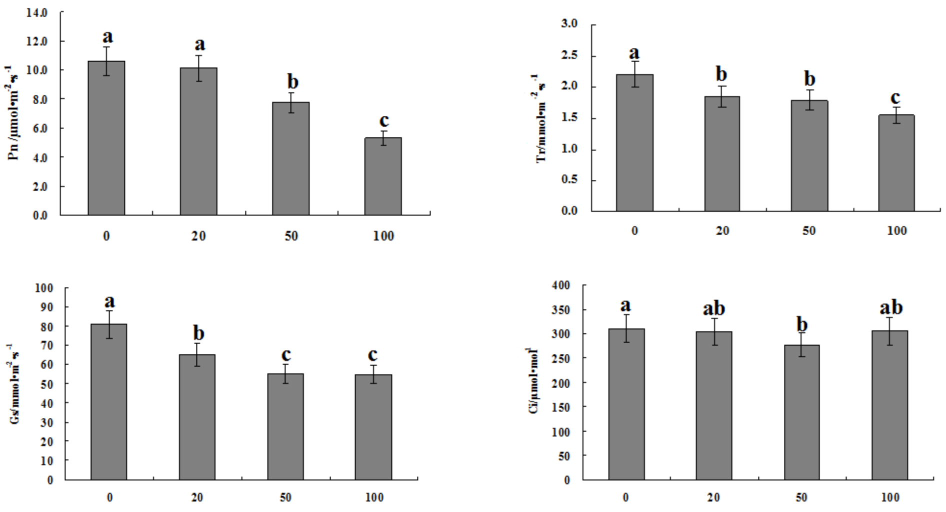

Figure 2. *Effects of Cd on the photosynthetic physiological indices of potato. The data are displayed as mean±SD, n=3. Different letters indicate the significant differences between treatments, P<0.05*

Effects of Pb and Cd on the chlorophyll content of potato

It can be seen from *Fig. 3* that the SPAD value of potato decreased with the increase of Pb and Cd concentrations, and the variation patterns were different. Under the Pb stress, the SPAD value showed a downward trend with the increase of the Pb concentration, and the decrease rate became smaller. When the Pb concentration exceeded 500 mg·kg^{-1}, the SPAD value tended to be stable. Under the Cd stress, the SPAD value decreased with the increase of the Cd concentration, and when the concentration of Cd exceeded 50 mg·kg^{-1}, the decrease of SPAD value became much faster.

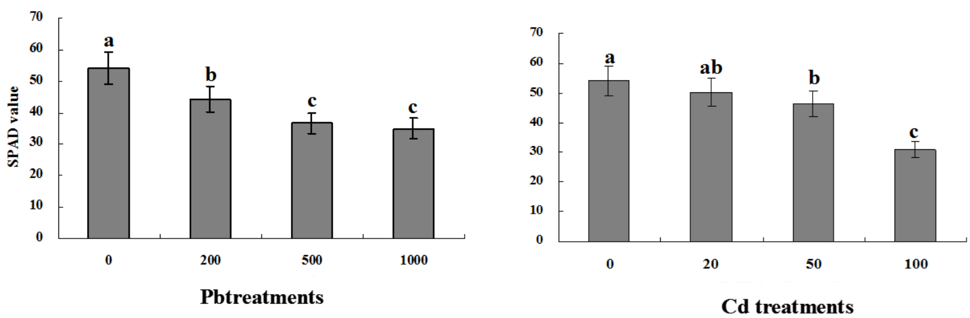

Figure 3. *Effects of Pb and Cd on the photosynthetic physiological indices of potato. The data are displayed as mean±SD, n=3. Different letters indicate the significant differences between treatments, P<0.05*

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Discussion

The photosynthesis of plants mainly depends on three physiological processes, namely, the conduction, light and dark reaction of the photosynthetic substrate CO_2. The leaves have strong CO_2 conductivity, and high light and dark reactions are the important foundations for plant leaves to achieve a higher photosynthetic rate (Liu et al., 2007; Yang, 2012). Environmental stress has multiple impacts on the photosynthesis of plants. It not only directly causes damages to the photosynthetic structure, but also affects the photosynthetic electron transport, the photosynthetic phosphorylation and the dark-reaction-related enzyme systems. It is generally believed that the factors causing a decrease of the photosynthetic rate include stomatal limitation and non-stomatal limitation (Jing et al., 2018). The net photosynthetic rate (Pn) of plant leaves is an important indicator to measure the photosynthesis of plants, and its changes can also directly reflect the degree and variations of photosynthesis. Factors affecting Pn mainly include Ci, Gs and Tr. They work synergistically during the plant photosynthesis to promote the photosynthetic reactions. Tr can measure the transpiration intensity and stomatal opening of plant leaves, of which the latter can directly affect the velocity of CO_2 entering the leaves and the intercellular CO_2 concentration, and thus affecting the carbon assimilation (Shabbir et al., 2016).

In recent years, the inhibition of plant photosynthesis by heavy metal has been confirmed by many studies. It is generally believed that the inhibition mechanisms include (Quartacci et al., 2000; Mukhopadhyay et al., 2015): heavy metals can destroy the photosynthetic enzymes in leaves and cause photosynthetic pigments to decrease; heavy metals reduce the chlorophyll content and cause the structural damages of the chloroplast and destruction of the chloroplast membrane. Chlorophyll is the initiator of photosynthesis. Within a certain range, the higher the chlorophyll content, the higher the net photosynthetic rate. After the Pb treatment, the photosynthetic pigments of plants were damaged, especially chlorophyll a. Its content decreased rapidly after the concentration of Pb increased, and the inhibitory effect was even more obvious under high-concentration Pb stress (Wang et al., 2009). The reduction of the chlorophyll content is related to the damage of the enzymes for the synthesis of chlorophyll. Some research proposes that, after being absorbed by plants, heavy metal ions act on the SH part of the peptide chain of the chlorophyll synthase and change its normal configuration to inhibit the activity of the synthase and block the synthesis of chlorophyll (Qiu et al., 2006). In addition, according to some studies, heavy metal ions can make the chloroplast envelope disappear, resulting in irreversible damages of chloroplast (Kutschera, 2015; Soares et al., 2016); under the Pb stress, the chloroplast structure will change significantly (Bashmakov et al., 2017); and under the high-concentration Pb stress, the chloroplast membrane system will collapse and the chloroplast will shrink, resulting in numerous large lipid globules (Zhou et al., 2005). It shows that heavy metal can cause great damages to the function of chloroplast and significantly reduce the chlorophyll content of plant leaves.

Heavy metals inhibit plant photosynthesis by affecting the stomatal opening of leaves, especially cadmium and lead. In this experiment, under the treatment of high-concentration Pb and Cd, the decrease of Pn became significantly faster, Gs significantly decreased, and at the same time Ci increased. According to the study of Farquhar and Sharkey (1982) on the determination of stomatal limitation and non-stomatal limitation, the inhibition of potato photosynthesis by Pb and Cd in this experiment was caused by non-stomatal factors. It was not because the decrease of

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stomatal conductance resulted in insufficient CO_2 supply, but because the photosynthetic structure was destroyed, which inhibited the activity of the dark reaction enzyme and thus reduced the photosynthetic rate of the plant (Mobin and Khan, 2007; Huang et al., 2018).

Conclusions

This experiment studied the effects of Pb and Cd on the photosynthesis of potato. After the treatment of Pb and Cd within a certain range of concentration, the photosynthetic rate (Pn), stomalal conductance (Gs), transpiration rate (Tr), intercellular CO_2 concentration (Ci) and chlorophyll content (SPAD) of potato were on a downward trend. As the concentrations of Pb and Cd continued to increase, most of the photosynthetic physiological indices tended to be stable, indicating that the potato photosynthetic system has certain resistance to Pb and Cd. Under the high-concentration Cd treatment, the SPAD value decreased sharply, which indicates that the effect of Cd on potato photosynthesis is greater than that of Pb.

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