Physiological Response of Cosmos Bipinnata Cav under Lead Stress

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Abstract. In this paper, Cosmos bipinnata Cav was studied and analyzed. by comparing the growth of Cosmos bipinnata Cav under different Pd waste residue environments, it was concluded that Cosmos bipinnata Cav was affected under the stress of heavy metal Pd. In the experiment, mainly through the changes of soluble sugars, soluble proteins, malondialdehyde and so on in plants in Pd environment, and at the same time, based on finding out the types of changes, the law of stress influence of Cosmos bipinnata Cav in the waste residue of heavy metal Pd was analyzed and studied.

Keywords: Cosmos bipinnata Cav, Lead, Growth status.

1. Introduction

The important value of soil in human survival and development is self-evident. In China, with the development of the times and the prosperity of social economy, the construction of modern society makes the land use area increase, and the occupied and polluted area of the original soil is also increasing, especially the heavy metal pollution. Heavy metals, as one of the main pollution sources in soil, have obvious influence on soil moisture and organic matter, and their destructiveness is very prominent, which will not only affect the growth and development of plants [1], but also cause the residual toxins in their bodies to affect human body and other organisms [2].

Through visiting and investigating the lead waste residue sites around Xichang city. The results showed that Gramineae, Commelina Diffusa Burm.f. of Equisetaceae and Cosmos bipinnata Cav of Compositae were the most distributed. The existence of lead waste residue in soil will lead to the change of soil fertility and its physical and chemical characteristics, and the influence of the change on plants and animals is often reflected in the form of negative influence [3]. Many kinds of heavy metals in soil have been shown in research and investigation, which have exceeded the load of plants in terms of the tolerance limits of plants themselves, which will lead to the poisoning of plants by heavy metals, which will further affect the growth and reproduction of plants and destroy the stability of the growth system of plants themselves [4]. It is characterized by abnormal expression of soluble sugar, protein and various enzymes. Investigation shows that the soil environment ultimately determines the growth, development and reproduction of plants. Through our field observation and statistics, there are still many kinds of plants that can successfully complete their life cycle in the lead waste field and show strong resistance to lead poisoning. In related reports [5], Cosmos bipinnata Cav has strong tolerance

to heavy metal pollution, and is an excellent ornamental flower, which is used for greening construction of scenic spots and has strong adaptability and barren resistance.

In this paper, through the formula of soil weighting metal, the pot simulation method was carried out to explore the influence of various physiological indexes and enzyme activities of Cosmos bipinnata Cav. With the method of heavy metal formula, we can deeply understand the tolerance of Cosmos bipinnata Cav to heavy lead ions and the ability of vegetation to repair soil, which provides powerful theoretical and experimental support for the research and analysis of Cosmos bipinnata Cav and other similar plants.

2. Materials and Methods

2.1. Test material

Use the purchased qualified Cosmos bipinnata Cav seeds, and use the aseptic seedling raising method to cultivate pollution-free, healthy and strong flower seedlings; Cosmos bipinnata Cav seedlings with similar plant height (5cm) and growth were selected and transplanted into the soil samples of formula for pot experiment. 5 plants were planted in each pot for 3 repetitions, and a group of blank control; Physiological tests were carried out after the plants had grown and blossomed for 3 months.

Planting five baht in each pot was repeated for three times, and another blank control group was selected as the reference for later research. After three months, physiological tests of each group were compared.

2.2. Experiment design

The pot soil used in the experiment is taken from the specific soil used in the experimental land. The test soil is mixed according to the test formula (Table 1) by adding lead ions, and each pot soil is mixed with 2 kg; This experiment is a one-factor variable experiment. There are 4 treatments in total, each treatment is repeated 3 times, and a blank control group CK is set.

Table 1. Soil preparation for Cosmos bipinnata Cav test

Soil formula of Cosmos bipinnata Cav test	
Test No.	Lead waste land soil (mg/kg)
T1	50
T2	100
T3	150
T4	200
CK	0

2.3. Test drugs and instruments

Drugs:Anhydrous ethanol、glacial acetic acid、sodium thiosulfate、trichloroacetic acid、30% hydrogen peroxide、DL methionine、L-proline、silicon dioxide、Ninhydrin、50% glutaraldehyde、Ammonium molybdate (tetrahydrate)、L-proline (chromatographic purity), sodium dihydrogen phosphate, Coomassie brilliant blue, cadmium chloride, 2.5 water phosphate, 2-thiobarbituric acid, hydroquinone, bovine serum protein, nitrotetrazolium chloride blue, hydrofluoric acid, etc.

Instruments: Three-purpose water tank, electrothermal constant temperature drying oven, visible light spectrophotometer, and table-type freezing centrifuge.

2.4. Detection Indicator

2.4.1. Extract enzyme solution. Cosmos bipinnata Cav was cleaned and washed with sterile water for 5 times. The research blades were ground and placed evenly. Then, a small amount of phosphate buffer solution with pH 7.8 and a small amount of quartz sand were poured into the condensed mortar according to the standard of 0.3g each, and then ground to slurry. The mortar was cleaned with buffer solution, and the volume of slurry was 6ml. Then, the grinding fluid used in the experiment was put into a centrifuge for treatment, with 15000 g at 4℃ as the experimental treatment index for 10 minutes. Then, the supernatant after centrifugation was taken and stored in a refrigerator at 4℃ for testing the experimental indexes.

2.4.2. Determination index and method. Determination method of catalase (CAT): hydrogen peroxide method, 50ul enzyme solution and 3mlCAT reaction solution were compared at 240nm, and the enzyme activity was measured every 1min for 5 times, and the decrease value of absorbance per minute was used to express the enzyme activity.

Soluble protein was determined by Coomassie brilliant blue staining.

Measuring method of MDA content and soluble sugar: take 1ml supernatant and mix it with 1mlTBA, react in boiling water bath for 15min, cool it quickly and centrifuge it, take supernatant and measure absorbance at 532,600nm.

2.4.3. Data processing and analysis. During the testing process, record the data well, manage it with Excel after testing, and finally calculate the standard deviation and variance with SPSS 20.0 software for statistical analysis.

3. Results and Analysis

3.1. Effects of different concentrations of lead on the catalase (CAT) activity of cosmos bipinnata Cav

It can be seen from fig. 1 that CATalase (cat) in Cosmos bipinnata Cav increases with the increase of lead content in soil, showing a trend of first increasing and then decreasing. Compared with CK group, CAT increased by 54.47%, T1 increased by 42.54%, T3 increased by 48.82% and T4 increased by 46.57%.

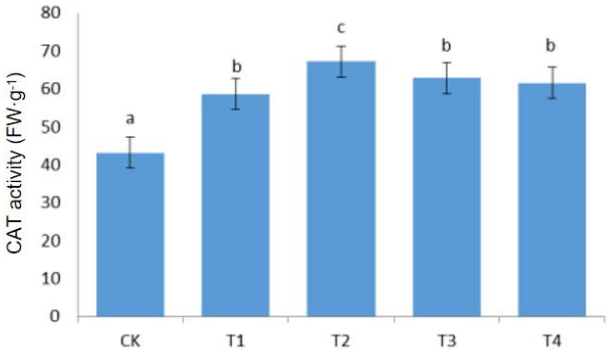

Figure 1. Activity diagram of CAT (FW/g)

3.2. The effect of soil with different concentration of lead on soluble protein content in Cosmos bipinnata Cav

There are many soluble proteins in plants, so they are often used as one of the main reference indexes for studying physiological metabolism of plants. Soluble protein in plants is a component of a large number of intracellular enzyme substances, which participates in a large number of physiological

metabolic processes of plants and affects many processes from growth and development to aging metabolism. Many diseases and stresses of plants are closely related to soluble protein in plants.

With the standard curve and linear equation of soluble protein, the soluble protein content of cosmobip innata CAV in soil with different concentrations of lead waste residue can be calculated (fig. 2).

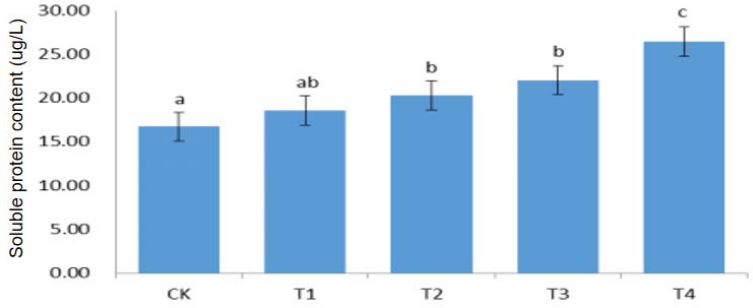

Figure 2. Change of soluble protein content (ug/L)

It can be easily seen from fig. 2 that with the increase of lead content in soil, the soluble protein in leaves of Cosmos bipinnata Cav also increases with the increase of lead content, and the changes of the two contents are in direct proportion, and the increase of soluble protein is significant in comparison between the experimental group and the control group. Compared with the control group, the protein content of the experimental group is nearly 50% higher, so it can be basically judged that the increase of stress protein is the reason for the increase of soluble protein content in plants after the increase of lead content in soil.

3.3. Changes of MDA content and soluble sugar

Through experimental observation, it is found that malondialdehyde is one of the main oxidation products of plant cell membrane, which can react with many internal components in cells, which will lead to the destruction or even necrosis of the corresponding enzymes and membrane structure. In the experiment, due to this factor, the content of malondialdehyde is often used as an index to measure membrane damage.

It can be easily seen from fig. 3 that the MDA content in Cosmos bipinnata Cav changes under different soil lead contents. The increase of lead content is directly proportional to the increase of MDA content. In the experiment, T4 reached the maximum value after treatment, and the difference was most significant compared with the control group ($P<0.05$). The content of MDA in vivo increased by 116.74% compared with the control group.

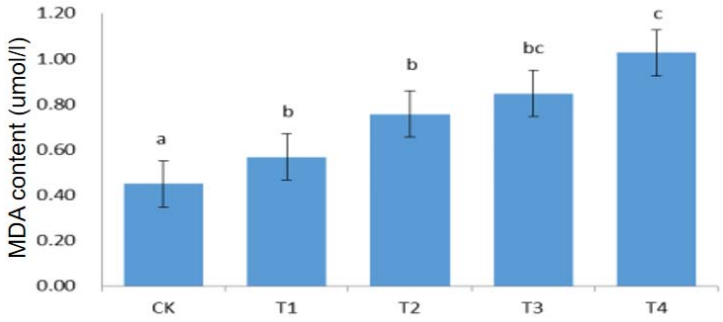

Figure 3. MDA content change (umol/L)

Soluble sugar is closely related to various metabolic reactions in plants, and the main action mode in plants is to regulate infiltration. According to the data in fig. 4, with the increase of lead content in the soil, the soluble sugar content in Cosmos bipinnata Cav also changed. at T3 treatment, the soluble sugar content was the highest, which increased by 32.13% compared with the control group.

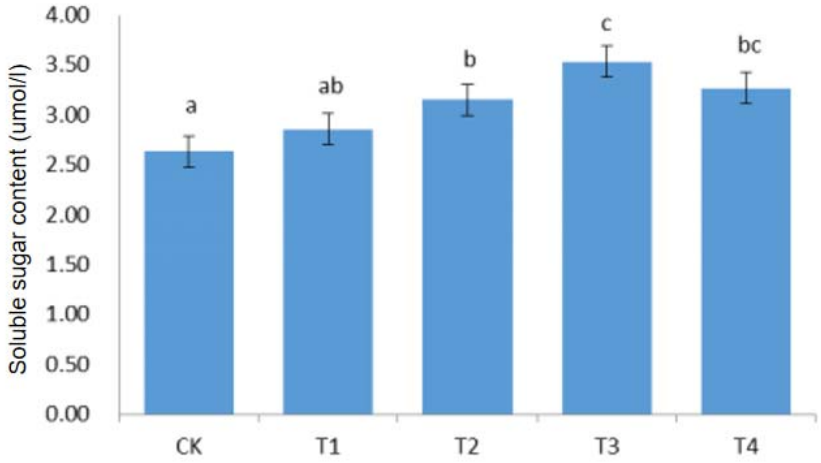

Figure 4. Change of soluble sugar content (umol/L)

Soluble sugar decreased during the experiment, and it decreased by about 7% in T4 group compared with T3 group. On the whole, the content of soluble sugar was not much different, but compared with the control group, the content of soluble sugar was quite different ($P<0.05$). Under such circumstances, it is beneficial to the growth and development of Cosmos bipinnata Cav under the environmental stress of Pd heavy metals.

4. Discussion

4.1. The effect of soil with different concentrations of lead waste residue on soluble protein content in Cosmos bipinnata Cav

The main reason is that the presence of heavy metals in the soil causes changes in the environment to release corresponding stress signals [6], which leads to different gene expressions of Cosmos bipinnata Cav, which further leads to changes in proteins in Cosmos bipinnata Cav [7], the new stress protein can help Cosmos bipinnata Cav to resist stress in heavy metal soil environment, thus improving the survival ability of Cosmos bipinnata Cav in heavy metal environment.

Under adverse conditions, the actual content of soluble protein in solution will decrease with the interference of environment [8]. In this process, macromolecular substances such as proteins will be degraded in different degrees, and the activity of enzymes, which are mainly composed of proteins, will also decrease when proteins are damaged. However, from the actual situation, under the adversity, the transformation process of many normal protein genes in plants will turn to activate the expression of more adversity genes, thus producing more adversity proteins to confront and adapt to the adversity environment. Under this stress condition, the decrease of normal protein can better maintain and stabilize the necessary physiological metabolism of cells to a certain extent, for example, by transforming the insoluble part of protein into soluble protein, which may be the reason for the increase of the proportion of soluble protein in the experiment to a certain extent.

4.2. Effects of soil with different concentrations of lead waste residue on MDA content and soluble sugar in Cosmos bipinnata Cav

The main component of cell membrane is phospholipid bimolecular, which often exists in the form of semi-permeable membrane, so as to better maintain the transport and exchange of cellular materials,

which is the component produced by malondialdehyde (MDA) after peroxidation. It can be inferred from the experimental data that the content of malondialdehyde increased, presumably because the heavy metals in the soil of lead waste residue destroyed the integrity of the cell membrane of Cosmos bipinnata Cav leaves. In this case, the permeability of Cosmos bipinnata Cav cell membrane tends to increase, which leads to the imbalance of water in leaf cells due to the change of permeability, which has a significant impact on normal metabolic activities. Especially under the interference of heavy metals, the active oxygen in leaves of Cosmos bipinnata Cav will accumulate more and more with the increase of accumulation time, which will further affect metabolism [9]. With the increase of the proportion of lead waste land soil, the MDA content in T4 treatment was the highest. It was speculated that the high concentration of heavy metals poisoned the soil environment, which made Cosmos bipinnata Cav severely persecuted, thus increasing the MDA content in leaves of Cosmos bipinnata Cav.

When plants are in an unfavorable environment where heavy metals exceed the standard [10], they will accumulate too much soluble sugar and soluble protein in the body, through which they can achieve osmotic adjustment in the body. In addition, a lot of carbohydrates are produced in this process, which can provide energy to plants and help them resist adverse environment, so as to regulate the growth and development of plants. Under the stress of heavy metals, Cosmos bipinnata Cav produces hydrolase, which accelerates starch hydrolysis and increases the sugar content in Cosmos bipinnata Cav cells for normal metabolic activities. According to the test results, with the increase of Pd waste residue in soil, the sugar content in Cosmos bipinnata Cav also increases, which makes Cosmos bipinnata Cav produce stress-resistant response.

4.3. Effects of different concentrations of lead waste residue on CATalase (cat) activity in Cosmos bipinnata Cav

Catalase (CAT) plays a very important role in plants, and it is also one of the enzyme substances with the highest content in the body. In plants, cells are often damaged by oxidation due to excessive H_2O_2 accumulation, which leads to programmed cell death. In this process, CAT removes H_2O_2 as a scavenger, which plays a significant role in plant stress resistance. By decomposing hydrogen peroxide, it is generated into oxygen and water, so as to reduce the damage of active oxygen to plant cells. With the increase of the proportion of lead waste land, the catalase (CAT) activity in Cosmos bipinnata Cav is enhanced, so the antioxidant capacity of Cosmos bipinnata Cav is enhanced, which can reduce the accumulation of active oxygen and is of great significance for Cosmos bipinnata Cav to resist adversity stress. However, the proportion of lead waste soil increases, the concentration of heavy metals is too high and long-term stress occurs. The situations of T3 and T4 are mainly beyond the tolerance range of Cosmos bipinnata Cav under stress environment, so the catalase activity in its body decreases compared with the normal growth demand. Especially, compared with T2 test group, its enzyme activity decreased obviously, which also reflected the characteristics of plant stress environment [11].

5. Summary

Pd is an essential element for the normal growth of plants, but its demand is not high, exceeding a certain range, and the tolerance of plants will not be able to adapt to and metabolize excessive Pd, while the content of lead exceeds the standard. In the growth and development of plants, it will cause damage to plants by hindering their metabolism. It can be seen from the experimental analysis that with the increase of Pd concentration, the MDA content in Cosmos bipinnata Cav also increases. It can be seen from the experiment of T4 group that Cosmos bipinnata Cav has been obviously affected by the stress of heavy metal soil.

In the range of normal stress resistance, Cosmos bipinnata Cav can adjust the changes of related enzymes and proteins by self-adjustment, so as to adapt to normal growth and development. It can be easily seen from the experimental data that under the influence of Pd, the activities of soluble protein, active oxygen and corresponding enzymes in Cosmos bipinnata Cav are obviously increased, and the

increase of soluble sugar is also obvious, which can be considered as the adjustment of substances in Cosmos bipinnata Cav in order to adapt to the survival of lead waste residue. However, the degree of environmental stress exceeds a certain limit, and cosmos bip innerata CAV will not be able to adjust related enzymes and proteins to meet its own growth. With the development of time, the cell structure in cosmos bip innerata CAV will be destroyed, thus affecting its own life process.

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