Research on the Mechanism of the Determination of Heavy Metal Tolerance of the Liliaceous Plant Root Elongation under the Special Environment

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Abstract
With the rapid growth of China's economy, the problem of environmental pollution has become one of the main problems in China's economic development. Influenced by industrial production and mineral exploitation, the contaminated soil in China is more and more, due to the high content of heavy metal in contaminated soil, most of the plants don't have enough heavy metal tolerance to survive, while liliaceous plants can still grow in a certain heavy metal content of the soil. Therefore, the heavy metal tolerance of liliaceous plants was determined by the root elongation of which in different polluted degree of the soils, so as to apply to the treatment of soil remediation. In order to more accurately obtain the experimental results, Ophiopogon japonicus was taken as research object, four kinds of soils collected in different places were compared, and the contents of heavy metals in the four groups were different, so the Pb, Cu, Cd, As, Hg were determined and analyzed. According to the classification standard of soil quality, the pollution levels were 1, 3, 4, 5 level, respectively. The experiment time was 2 weeks, in addition to the quality of growth soil was not the same, the rest of the growth environment was the same. After the end of the experiment, the number of root, the longest root length and average root elongation, root shoot ratio and other parameters were analyzed respectively. It can be seen from the experimental results that when the pollution level is 3 and below, Ophiopogon japonicus can still grow, in the moderate polluted and even severe polluted environment, Ophiopogon japonicus can't grow, and will be affected by the impact of heavy metals and gradually die.

Keywords: liliaceous plant; root elongation; heavy metal; tolerance; soil quality.

Introduction

Soil pollution is one of the serious consequences of the ecological environmental protection in the process of economic development in China, which not only affects the use of arable land in China, but also causes the destruction of forest land and grassland, and thus affects the ecological balance of a certain region [1]. The most serious is the heavy metal pollution in soil pollution, heavy metal pollution is mainly influenced by the industrial waste water discharge and the rejected material, among them, a large number of heavy metals deposit to the soil, so as to increase the contents of heavy metals in the soil, when the content exceeds a certain value, the plants can't grow and the soil surface vegetation is destroyed. Although China has attached great importance to the remediation of the soil, but because the research on the technology of remediation is absent, and investment is not enough, so that the governance effect is not ideal [2]

As a common plant species in China, liliaceous plant is widely grown in various regions of China, its types are many, and it is wildly used in people's daily life. Through the previous studies, it can be found that the liliaceous plant has some heavy metal tolerance, so it can be grown in contaminated soil [3]. Therefore, there is not a relatively accurate research conclusion that what kind of soil pollution levels can make liliaceous plant continue to grow, so that which is not conducive to the actual production practice. And Ophiopogon japonicus in liliaceae plants not only has very high medicinal value, but also grows in people's living environment as a green ornamental plants, it is a kind of common liliaceae plant in daily life. Because of the contaminated soil, the first influenced part of plant is the root, while the growth of root directly determines the whole plant growth, so the heavy metal tolerance can be analyzed through the plant root elongation. In this paper, Ophiopogon japonicus was selected as the research object to analyze the growth of root elongation in the polluted soil, and the heavy metal tolerance was determined, so as to obtain the growth of Ophiopogon japonicus in different pollution levels of soils, which has a certain reference for the actual production of contaminated soil remediation management. [4]

Soil heavy metal pollution and heavy metal tolerance of liliaceous plant

Soil heavy metal pollution: With the development of industry and agriculture and the rapid development of economy in China, the environmental pollution has become more and more serious.

This will not only seriously affect people's living environment the contaminated soil will directly affect the growth of the plants [5]. The soil is mainly polluted by heavy metals, when the heavy metal content exceeds the self purification capacity of the soil, soil quality will decline, plants will grow slowly, and even harder to live, even if it is able to survive, compared to the normal soil, the heavy metal content in each part of the plant is high, which greatly reduces the utilization value of the plant [6]. At present, China's soil pollution can be divided into industrial pollution sources, agricultural pollution sources, life pollution sources and other pollution sources of the four major categories, pollution includes inorganic and organic pollution, heavy metal pollution belongs to inorganic pollution, including lead, arsenic, cadmium, copper and so on [7]. As an agricultural country in the world, China's cultivated land is very important, however, according to statistics, 150 million mu of cultivated land in China is polluted by heavy metals, which accounts for 8.3% of the arable land in China. In these contaminated land, the cultivated land with moderate pollution is difficult to plant grain, even the grain is planted in lightly contaminated arable land, there are different levels of heavy metal contents in the harvested grain, which is a threat to people's lives and health [8]

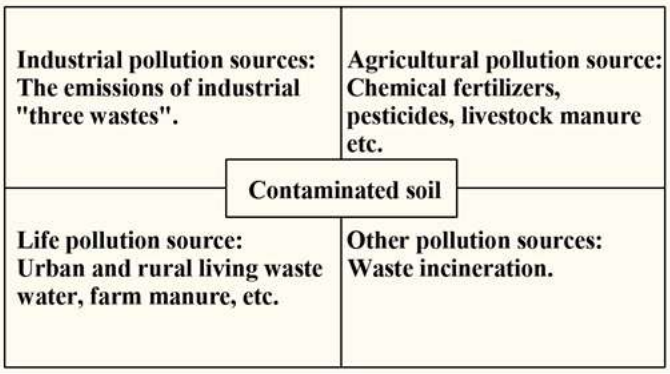

Fig. 1: Pollution source classification

At present, China has already had the concrete standard request to the soil environmental monitoring. The safety assessment of heavy metal in soil uses the single pollution index method and the comprehensive pollution index method of the two models [9] Among them, the formula of single pollution index is:

$$P_i = \frac{C_i}{S_i} \tag{1}$$

Among them, P_i is the single pollution index, C_i is the measured data of pollutants, S_i is the evaluation criteria. According to the results of the calculation, when P_i is greater than 1, it indicates that the soil is polluted, the greater the value, the more serious the pollution; When P_i is less than 1, it shows that the soil is not affected by the pollutants.

P_i can only represent the effect of a single pollutant on the soil quality, but in practice, the heavy metal pollution of the soil is not affected by a single pollutant, the comprehensive pollution index should also be evaluated, the calculation formula is:

$$P_{comp} = \left[\frac{1}{2} (P_{\max}^2 + P_{Aver}^2 \right]^{\frac{1}{2}} \tag{2}$$

Among them, P_{comp} is the comprehensive pollution index, $P_{\max}$ is the maximum value of the single pollution index, P_{Aver} is the average of the single pollution index arithmetic mean. According to the comprehensive pollution index of the soil, the soil quality grade is evaluated, and divided into 1, 2, 3, 4, 5 level, the specific criteria of the division is:

Table 1
Classification evaluation of soil environmental quality

Pollution index	**Soil grade**	**Pollution assessment**
P≤0.7	1	Safety grade
0.7<P≤1	2	Alert level
1<P≤2	3	Light pollution
2<P≤3	4	Moderate pollution
3<P	5	Heavy pollution

Heavy metal pollution is likely to cause the loss of "life" to the soil, and most of the plants are difficult to survive in contaminated soil. And because the heavy metal is very difficult to decompose in nature, it is difficult to deal with it [10]. Today, the treatment measures of heavy metal contaminated soil mainly include the electrochemical method, heat modification treatment, washing complexation method and other engineering measures, as well as phytoremediation, microbial remediation and animal repair and other biological remediation measures, in addition, there are also chemical treatment measures, among these treatment measures, although the cycle of the biological remediation measure is long, however, the efficiency is high and the cost is low, so that which is widely used in practice [11]. In bioremediation treatment, the most commonly used is the plant repair measure, which can be widely used in mine reclamation, heavy metal contaminated soil improvement, industrial waste water purification, etc.. When phytoremediation is carried out, it is necessary to select plants with a certain tolerance to heavy metals, so that the plants can survive and improve the pollution of the soil in soils contaminated by heavy metals[12].

The heavy metal tolerance of liliaceous plant: Liliaceous plants are distributed all over the world, and mainly distributed in temperate and subtropical regions. And the geographical position of China is in the suitable area of liliaceous plants. According to statistics, there are about 560 species of liliaceous plants in China. Liliaceae plants belong to perennial herbaceous plants, have rhizomes or tubers, bulbs, leaves basal or cauline, stamen is generally 6 pieces with abundant endosperm.[13]

Fig. 2: Heavy metal contaminated soil

From the use value, liliaceae plants have an important economic value, which can be used for greening, eating, watching, and so on. Asparagus, Ophiopogon root, Rhizoma Paridis, Rhizoma Anemarrhenae, Polygonatum odoratum and others are the commonly used traditional Chinese medicine herbs, and widely planted by people; Onions, lily, garlic and other daily foods belong to the liliaceae plants, which are the common ingredients and even the essential ingredients in our lives; The ornamental plants such as the Tulip flowers, Chlorophytum are also loved and commonly used by the people for the green or decoration [14]. And the liliaceae plants are not just a single use, in the case of Ophiopogon japonicus, which not only is the famous Chinese herbal medicine, but also is commonly used in food plants and the production process. In the remediation of contaminated soil, the liliaceae plants become one of the commonly used plants because of its strong heavy metal tolerance [15]

Because of the contaminated soil, the first influenced part of plant is the root, so the determination of root elongation can reflect the heavy metal tolerance of plants to some extent [16]. According to the existing research, because of the effect of Cu, liliaceae plants can be inhibited by the concentration of the solution with the increase of the concentration of copper, so that the plants become shorter. But compared to other conventional plants, the tolerance of the liliaceae plants is strong to heavy metals, and it can be used for the remediation of contaminated soil in copper mine area [17]. The tolerance of plants to heavy metals is expressed by the tolerance index, its calculation is:

$$\text{耐性指数} = \frac{\text{植物在重金属污染土壤中的最长根长的平均值}}{\text{植物在常规土壤中的最长根长平均值}} \times 100\% \qquad (3)$$

Based on the tolerance index, research on the heavy metal tolerance of plants can be carried out through the root length, the larger the tolerance index, the better the heavy metal tolerance, otherwise, it shows that the growth of the plants is inhibited by heavy metals, and even can't grow, so that the plants are destroyed by the heavy metals.

Fig. 3: Lily plant

Experimental materials and methods: In this experiment, the representative of liliaceae plants Ophiopogon japonicus was selected as the research object because of its wide range of use and the relatively high use value, which was distributed in all parts of China. In order to more objectively reflect the experimental results, the Ophiopogon japonicus selected was divided into A, B, C, D in four different groups, but the growth state and the growth cycle of Ophiopogon japonicus were almost the same, the difference of root growth and the ground floor of the plant was not large.

A, B, C, D four groups of experimental soils were obtained from the outdoor, among them, the soil in group A was not significantly contaminated, the soils in group B, C, D were contaminated in different degrees, the soil sampling site was a city in Sichuan, the specific sampling site of B, C, D soil was in the industrial waste emissions surrounding contaminated areas of the city. After obtaining enough soil samples, the analysis of heavy metal elements in four kinds of soils was carried out, including the heavy metal content analysis of Pb, Cu, Cd, As, Hg of these five kinds. The specific circumstances are shown below:

Table 2
Statistics of heavy metal content in soil (mg/kg)

Team	Pb	Cu	Cd	AS	Hg
A	13.5	21.9	0.114	1.44	0.088
B	39.6	41.6	0.303	18.8	0.247
C	36.4	48.4	0.498	32.3	0.295
D	23.6	34.5	0.751	6.82	0.260

According to formula (1), (2), the individual and composite index of the group A, B, C, D were calculated and analyzed. The heavy metal single pollution index is as follows:

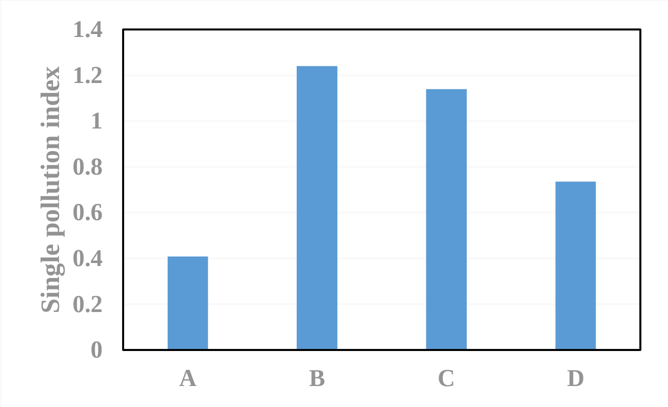

Fig. 4: Pb Single pollution index

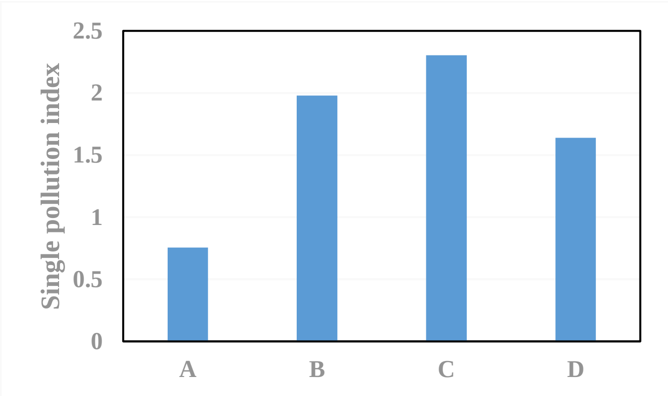

Fig. 5: Cu Single pollution index

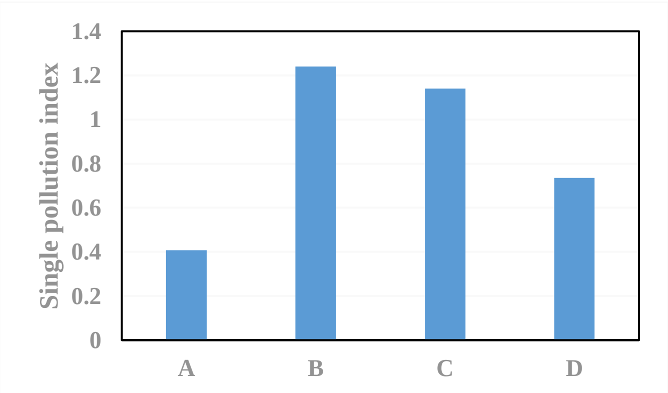

Fig. 6: Cd Single pollution index

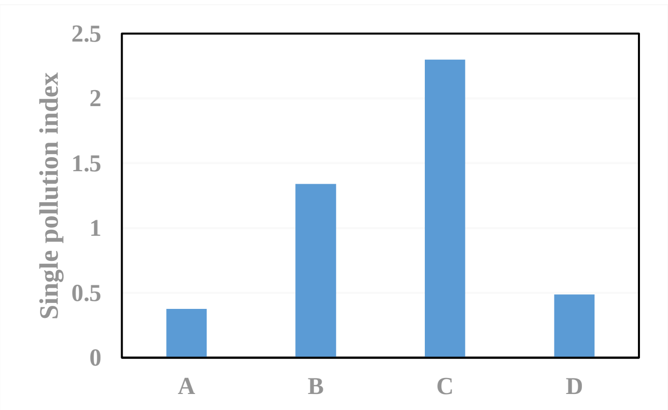

Fig. 7: As Single pollution index

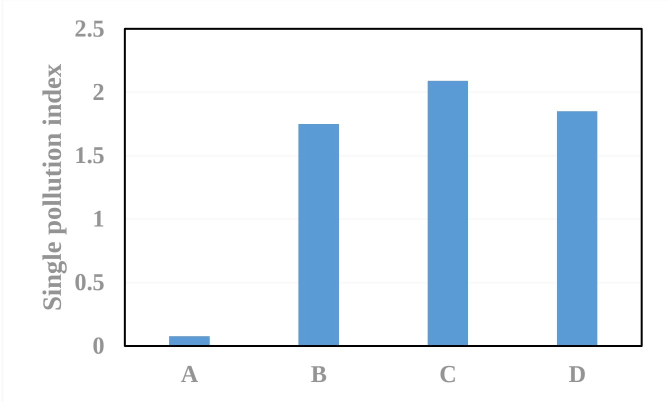

Fig. 8: Hg Single pollution index

From the Figure 4 to Figure 8, it can be seen that the pollution of heavy metals in each group was not the same, the Cd single pollution indexes in group B, C, D group were relative high. The comprehensive pollution indexes are as follows:

Table 3
Heavy metal pollution assessment

Team	**Single pollution index**					**Comprehensive pollution index**	**Class of pollution**
	Pb	**Cu**	**Cd**	**AS**	**Hg**		
A	0.408	0.755	0.586	0.377	0.078	0.618	1
B	1.24	1.98	1.76	1.34	1.75	1.81	3
C	1.14	2.304	2.86	2.30	2.09	2.53	4
D	0.736	1.64	4.32	0.487	1.85	3.31	5

Through the detection of the soil, it can be seen that the soil pollution indicator in group A was 0.618, less than 0.7, so its environmental quality level was 1, was the safe and clean type. The comprehensive index of soil pollution in the group B was 1.81, and the environmental quality grade was 3, which belonged to the mild pollution. The soil environmental quality grade of group C was 4, which belonged to moderate pollution. The soil environment quality grade of D group was 5, which belonged to heavy pollution [18]

The experimental results were reflected from the tiller number of root, total root number, the longest root length, average root growth elongation and the ratio of root to shoot ratio of the several aspects. Among them, the number of tiller refered to the branches of the plant under the ground, the number of tiller number was affected by the temperature, light, soil environment and other aspects of the impact, which could reflect the growth of plants. The calculation formula of root cap ratio is as follows:

$$\text{Root shoot ratio} = \frac{\text{Fresh weight of the underground part of the plant}}{\text{Fresh weight of the above ground parts of the plant}} \quad (4)$$

The root shoot ratio could reflect the correlation of underground and above ground parts of the plant, in good growth environment, the underground root growth rate of the plants was fast, the ground part of the plants also grew.[19]

The experiment was carried out for two weeks and 14 days, during the experiment, the soils in the group A, B, C and D were not the same, the temperature, light, moisture, humidity and other external environment were consistent. After two weeks, the growth of the Ophiopogon japonicus in four groups were measured and counted, and then the comparative analysis was carried out, so that the growth condition of Ophiopogon japonicus under different soil environment was studied, and the further analysis of the heavy metal tolerance was carried out.

Experimental results and analysis

Experimental results

Through 14 days of cultivation, it can be found that Ophiopogon japonicus in group A and group B grew better in the four groups of Ophiopogon japonicus test from the appearance of plant growth, Ophiopogon japonicus in group C stoped the growth, even withered signs, while groupD was completely dead. Through the determination of further growth of Ophiopogon japonicus in each group, it can be seen that: The number of tillers and root total rooting in group A were 4, the average root length was 3.12cm, the root shoot ratio was 0.42; The number of tillers and root total rooting in group B were 4 and 3, the average root length was 2.78cm, the root shoot ratio was 0.38; The number of tillers and root total rooting in group C were 1 and 1, the average root length was 0.21cm, the root shoot ratio was 0.14; The number of tillers and root total rooting in group D were 0 and 0, the average root length was 0cm, the root shoot ratio was 0.03. The growth of Ophiopogon japonicus in each group is shown in the table 4.

Through the further analysis, it can be seen that Ophiopogon japonicus grew well in group A and group B, and the soil quality grades of group A and group B were the 1 security level and 3 levels of light pollution, respectively, which indicated that even the soil was slightly polluted, Ophiopogon japonicus still continued to grow. But compared to group A, The average root elongation, root elongation and root shoot ratio value of group B were smaller than that in group A, which indicated that although the growth of Ophiopogon japonicus in group B group was good, but which was still not as good as the growth of Ophiopogon japonicus in group B.

The average maximum root elongation of group C was 0.41cm , root elongation was only 0.21cm, it can be initially inferred that it had a certain growth in the first few days, but was contaminated by the moderately polluted soil environment. Ophiopogon japonicus in group D almost had no growth, heavily polluted soil made the Ophiopogon japonicus dead, in this soil environment, the Ophiopogon japonicus couldn't grow.

Table 4
Statistical analysis of experimental results

Grouping	Tiller number of rooting	Total rooting number	The longest root /cm	Average root length/cm	Root shoot ratio
A	4	4	3.56	3.12	0.42
B	3	4	3.32	2.78	0.38
C	1	1	0.41	0.21	0.14
D	0	0	0.06	0	0.03

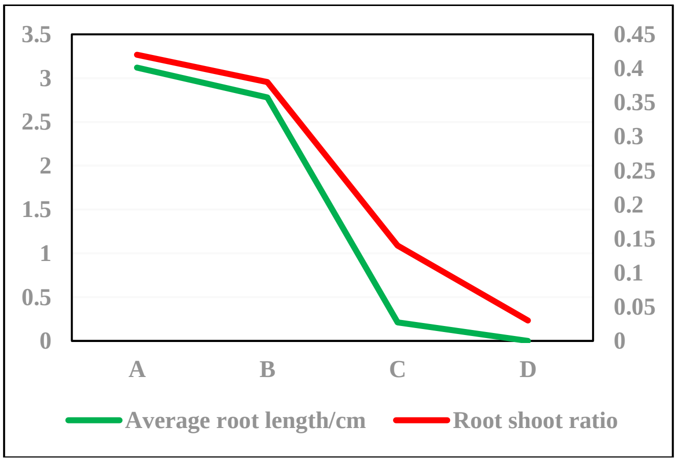

Fig. 9: The average root elongation and root shoot ratio curve

Through four groups of experiments it can be seen that the growth of Ophiopogon japonicus became poor with A, B, C, D in order, the effects of contaminated soil on the growth of Ophiopogon were relative large, resulting in the no growth of the plants. In the serious polluted soils, Ophiopogon japonicus growth stoped, and started from the roots, this indicated that when the plants were polluted by heavy metals in soil, the root was the first part to be affected (Jin G, et al. 2000) [20]. Which also indicated that the determination of the root elongation had an accurate judgment for the growth of the plants, so that the analysis of the metal tolerance was carried out.

Discussion and analysis

Through the comparison of the growth situation of Ophiopogon japonicus in four groups, the following conclusions can be drawn: (1) Soil in group A was not affected by the soil pollution, the growth of Ophiopogon japonicus was good, through the comparison of the growth of Ophiopogon japonicus in group A, the influence of soil pollution of Ophiopogon japonicus in other groups could be analyzed; (2) With the deterioration of soil quality and the rise of pollution level, the growth of Ophiopogon japonicus was inhibited, even in moderately polluted environment, the decay phenomenon of Ophiopogon japonicus occured; (3)

Under the mild polluted environment, although the growth of Ophiopogon japonicus was restrained, but it still grew well, which indicated that the heavy metal tolerance was relative good.

In group B soil, although its Pb and Cu contents were high, which had certain inhibitory effect on the growth of Ophiopogon japonicus, but the impact was not big, this also indicated that the soil Pb, Cu contents used in the experiment didn't reach the concentration of toxic effects on plants. The contents of Cd and Hg in soil were not high, but which had great influence on the growth of plants.

Through the analysis of experimental results, it can be seen that even under the lightly contaminated soil, Ophiopogon japonicus still can continue to grow, and compared to the non-contaminated soil, its growth is not affected. Therefore, Ophiopogon japonicus can be planted in the soil which is polluted slightly, so as to be used for greening and improving soil environment, which is conducive to improving the quality of the soil. And most of the regional climates in China are suitable for the growth of Ophiopogon japonicus, so it can be widely used in remediation of soil contaminated by heavy metals. At the same time, in soils contaminated by Cd and Hg, it is not suitable to use Ophiopogon japonicus for bioremediation, it is necessary to first consider the ways of governance in governance or chemical engineering.

The experiment also has some limitations and deficiencies, the soils used in the experiment are taken from the natural environment, therefore, the experiment lacks of adequate pertinence, the contents of heavy metals in the soil are not actively regulated by a man-made, and after sampling, the content of the soil is analyzed, and the soil is evaluated by the pollution level. When the effect of heavy metals on the growth of plants of Ophiopogon japonicus is analyzed, it is difficult to have a single plant specific analysis of the impact of heavy metals. At the same time, due to the limited number of groups, it is difficult to fully explain the heavy metal tolerance of liliaceae plants, so it is needed to further expand the experimental groups and the number of samples, and analyze different plants of liliaceae plants, so as to comprehensively analyze the metal tolerance of the plants.

Conclusion

Remediation of soils contaminated by heavy metals has become a difficult problem in environmental management in China in recent years. Because most of the plants are difficult to survive and grow in the soil polluted by heavy metals, plant repair methods are difficult, while in other ways, the cost is high, and it is not suitable for popularization and application. Through previous studies, it can be seen that the liliaceae plants can still survive and grow in a certain degree of contaminated soil. In order to further measure the metal tolerant, the Ophiopogon japonicus in liliaceous plants was seclected to carry out the comparative experiments in the soils with different quality. Experiment was divided into four groups, the soils used in the experiments were collected from different environment, and the pollution levels were not the same. In the experimental process, the remaining temperature, light, moisture, humidity and other external environment were consistent, after two weeks of culture time, the growth situation of Ophiopogon japonicus in four groups in the experimental were determined and analyzed, including the tiller number, root total root number, root elongation and root shoot ratio and so on. By comparing the experiments, it can be seen that although the growth of Ophiopogon japonicus is affected by the inhibition of heavy metals, but which can still grow well in the light pollution and the following degree of pollution in the soil. When the pollution level is moderate and above, Ophiopogon japonicus is difficult to continue the grow. Therefore, in the actual remediation of soil pollution, it is necessary to analyze the quality of soil pollution, and then choose the appropriate remediation measures according to the pollution level.

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