Study of the Physiological and Ecological Characteristics of *Peperomia Tetraphylla* Stressed on Phenol solution

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Abstract. The paper aims to study the change of physiological and biochemical indexes through simulating the effect of different concentrations of phenol solution on the growth of *peperomia tetraphylla*. Then explore the degree of intimidated, and reveals whether the *peperomia tetraphylla* could regard as the best plant to absorb the phenol and purify the wastewater. Chlorophyll and malondialdehyde (MDA) were regarded as the physiological and biochemical indexes. The results indicated that under the stress condition which the concentration of phenol is 100 mg/L, leaves of *peperomia tetraphylla* on the content of chlorophyll and MDA had both increased significantly, it suggests that under the concentration that *peperomia tetraphylla* has very good adaptability. Under the stress condition which the concentration of phenol is 80–200 mg/L, stem of *peperomia tetraphylla* on the content of MDA had increased.

1. Introduction

Phenol (C₆H₅O, PhOH), also known as carbolic acid, is the simplest phenolic organic matter with high toxic and carcinogenicity [1]. It can slightly soluble in water at room temperature and easily soluble in organic solutions. When the temperature is higher than 65°C, it can be miscible with water in any ratio. It is pink when exposed to the air. Phenol is a common chemical and an important raw material for the production of certain resins, fungicides, preservatives and drugs such as aspirin.

Phenol in the environment mainly comes from phenol-containing wastewater and waste gas discharged from coking, oil refining, gas production, phenol and its compounds, and industrial discharges which using phenol as raw materials. If untreated phenol-containing wastewater is irrigated through open channels, it will volatilize into the atmosphere or seep into the ground, polluting the atmosphere, groundwater and crops. Phenol will harm aquatic organisms when enters the water body, it can cause acute toxicity to fish and algae. It will cause obvious changes in the reproduction behavior of organisms, and then affect the entire water ecosystem. Its toxicity will accumulate in aquatic organisms and enter other organisms through the food chain. The polluted water body is difficult to be purified, which threatens the safety and health of human drinking water. At present, the environmental pollution caused by phenol should be paid special attention. Phenol has also been included in the blacklist of 129 priority pollutants by the US Environmental Protection Agency. Therefore, phenol is an important indicator of environmental monitoring [2].
Phenol and its compounds are moderately toxic substances. It can enter the human body through various ways such as skin, mucous membrane, respiratory tract and oral cavity. Phenol and its compounds are a kind of cell protoplasmic poison. The toxic effect in the body is to chemically react with the protein in the cell protoplasm to form denatured proteins and make cells inactive [3]. The pathological changes caused by phenol and its compounds mainly depend on their concentration. Low concentrations can denature cells, and high concentrations can coagulate proteins. Although the local damage of low concentration to human body is not as serious as that of high concentration, because of its strong penetrating power, it can penetrate into internal tissues, invade the nerve center, stimulate the spinal cord, and eventually lead to systemic poisoning [4]. Phenol has a strong corrosive effect on skin and mucous membranes, and can also inhibit the central nervous system or damage liver and kidney functions.

Phenol has a toxic effect on all living individuals and is included in the national hazardous waste list. The wastewater discharge standard limit of phenol solution is 0.5mg/L [5]. Phenol-containing wastewater is a common, difficult-to-treat, toxic and hazardous wastewater that requires high treatment standards. Phenol-containing wastewater treatment usually adopts methods such as adsorption [6], advanced oxidation, membrane separation, and biodegradation [7]. Among them, activated carbon adsorption and biodegradation are the most widely used methods.

Phytoremediation technology is a process of decomposing, enriching and stabilizing toxic and harmful pollutants in environmental media by using the absorption and metabolism functions of plants. Due to the different physical and chemical properties and environmental behaviors of pollutants, combined with different plant metabolisms, so the mechanism of phytoremediation of pollutants is not the same, but generally can be divided into plant fixation, plant degradation, plant volatilization, plant extraction and root filtration. Plant immobilization is the use of plants to absorb toxic and harmful pollutants such as heavy metals in the roots, stems, and leaves of plants, reduce their mobility and prevent them from spreading to the surrounding water. Plant degradation is the use of plants or plants and microorganisms to degrade organic pollutants. Plant volatilization refers to the volatilization of certain volatile pollutants from the gaps in the surface tissue of the plant after being absorbed by the plant. Plant extraction is the transfer of heavy metals or organic pollutants in the soil from the contaminated soil to the above-ground parts of the plant by plant roots. Root filtration refers to the use of plant roots to absorb and absorb organic pollutants in water or wastewater. It should be pointed out that the mechanism of action of plants on organic pollutants is often not a single way, but the result of two or more simultaneous combined actions, which increases the complexity of phytoremediation technology to a certain extent [8]. Since the 1990s, phytoremediation technology has become a frontier topic in the field of environmental pollution control research. It is a new, economic, effective, and non-destructive remediation method with great potential. Phytoremediation requires plants to treat pollutants with high adapt, otherwise the pollutants will be poisonous to plants, cause plant death or affect the normal growth of plants. Generally speaking, plants are suitable for repairing low or medium concentrations of pollutants. As a means of purifying polluted water bodies, aquatic plants have been recognized by scholars at home and abroad.

Green leaf peperomia tetraphylla is also called watercress green, pepper grass, and emerald pepper grass, and belongs to the pepper family. Watercress green is a perennial evergreen plant. Generally, peppergrass varieties with green leaves can grow well in weak light, have strong adaptability, strong shade tolerance, strong adaptability, can purify the air, decontaminate, absorb formaldehyde, and prevent radiation.

This study simulates the intervention of different concentration gradients of phenol solution on the growth of peperomia tetraphylla, studies the changes of physiological and biochemical indicators, and explores the degree of stress, thereby revealing whether peperomia tetraphylla can be an ideal plant for absorbing phenol and purifying sewage.
2. Experimental Details

2.1. Instruments and reagents
The equipment and materials needed for the experiment mainly includes UV-2550 ultraviolet spectrophotometer (Shimadzu Corporation, Japan), BSA124S electronic balance (Sartorius Scientific Instruments Co., Ltd.), constant temperature water bath, 28 transparent plastic buckets, pipette, plastic cling film, quartz cuvette, filter paper, mortar, ear ball, scissors.

Commonly used glass instruments in the experiment mainly contains 6 beakers, 2 glass rods, 4 volumetric flasks (100mL), 2 brown volumetric flasks (100mL), 35 colorimetric tubes with stoppers, and thermometers.

The reagents mainly contains Phenol AR (Laiyang Shuangshuang Chemical Co., Ltd.), distilled water, quartz sand, calcium carbonate, absolute ethanol, 5% trichloroacetic acid (TCA), 0.67% thiobarbituric acid (TBA).

2.2. Collection and cultivation of plant samples
The experimental materials were purchased from the flower market. 21 plants were selected to select fresh green, good-growing, similar plant heights and similar weights of *peperomia tetraphylla*. The *peperomia tetraphylla* was taken out of the flower pot and the soil on the plant was cleaned without damaging its roots. Incubate in clean water in the laboratory for 2 to 3 days, under the conditions of room temperature (16 ℃), 3000 lx light intensity, and 12 h: 12 h light-to-dark cycle ratio. After incubation, phenol pollution treatment is carried out.

2.3. Determination of the initial concentration of phenol solution
In the preliminary experiment, select the *peperomia tetraphylla* that has grown vigorously after domestication and culture (the size, thickness, and height are more consistent), put it on the filter paper to absorb the water on the surface, weigh 24 portions of 35 g of *peperomia tetraphylla*, and put them in different concentrations. (0, 20, 40, 60, 80, 100, 200 mg/L) of phenol treatment liquid in a 5L plastic bucket, covered with plastic wrap, and placed in the laboratory for cultivation. The conditions are set to room temperature and the light is fluorescent light strength.

2.4. Preparation of phenol solution
Weigh 20.000 g of phenol into a 50 mL beaker, add a small amount of distilled water to stir to dissolve, transfer to a 2000 mL volumetric flask, and rinse the beaker several times, dilute to the mark with distilled water, shake well, and seal with plastic fresh-keeping film Stopper the volumetric flask and store it in a cool place for later use [9]. In 28 transparent plastic buckets, pipette 0, 8, 16, 24, 32, 40, 80 mL of phenol stock solution, and add 4000, 3992, 3984, 3976, 3968, 3960, 3920 mL in sequence distilled water with a concentration of 0, 20, 40, 60, 80, 100, 200 mg/L phenol solution.

2.5. Cultivation and weighing of *peperomia tetraphylla*
Take out the *peperomia tetraphylla* growing in the water, put it on the filter paper to absorb the water, weigh the same similar *peperomia tetraphylla* (about 35 g), a total of 21 parts, put them into a series of phenol solutions of different concentrations, and cover them to keep fresh Membrane, set three parallel controls for each concentration, and observe the changes of *peperomia tetraphylla*. After 7 days, the *peperomia tetraphylla* grown in phenol solutions of different concentrations was taken out, placed on filter paper to absorb excess water from the roots and stems, and weighed on a platform scale.

2.6. Determination of chlorophyll content in *peperomia tetraphylla* leaves
According to the absorption of the chloroplast pigment extract to the visible spectrum, the extinction degree of the spectrophotometer is measured at a certain wavelength, and the formula can be used to calculate the content of each pigment in the extract. The wavelengths of the maximum absorption peaks of chlorophyll a and b in absolute ethanol are 665nm and 649nm, respectively.
Cₐ = 13.95D₆₆₅ - 6.88D₆₄₉  \tag{1}

C₇ = 24.96D₆₄₉ - 7.32D₆₆₅  \tag{2}

Chloroplast pigment content = pigment concentration (C) * extract volume * dilution factor / fresh weight of *peperomia tetraphylla* leaves  \tag{3}

After culturing for 7 days, take the leaves of each *peperomia tetraphylla* plant with similar shape and close color, wash and wipe off the surface dirt, cut into pieces (remove the midrib), weigh 0.2g respectively, put it in a mortar, and add a small amount grind quartz sand, calcium carbonate and 2~3ml of 96% ethanol into a homogenate, then add 10ml of ethanol to grind, let stand for 3~5min, filter, and dilute.

Pour the chloroplast pigment extract into the cuvette. With absolute ethanol as a blank, the extinction degree was measured with a UV-2550 ultraviolet spectrophotometer at wavelengths of 665, 649, and 470 nm. Calculate the concentration of chlorophyll a and b of *peperomia tetraphylla* according to formula \(\text{(1)}\) and \(\text{(2)}\), and add the two formulas to get the total chlorophyll concentration. After calculating the concentration of the pigment, press the formula \(\text{(3)}\) to calculate the content of each pigment in the black algae leaves [10].

2.7. Respective determination of MDA content in *peperomia tetraphylla* leaves and stems

When plant organs age or suffer damage under adversity, membrane lipid peroxidation often occurs. MDA is the final decomposition product of membrane lipid peroxidation, and its content can reflect the degree of damage to plants. MDA is a commonly used indicator of membrane lipid peroxidation. Under acidic and high temperature conditions, it can react with thiobarbituric acid (TBA) to form a red-brown trimethyl complex (3, 5, 5').-Trimethylloxazole-2, 4. dione), its maximum absorption wavelength is 532nm. The absorption coefficient of the complex is 155 [mmol/(L · cm)], and there is minimal light absorption at a wavelength of 600nm. According to the formula:

\[ A_{532} - A_{600} = 15000 \times C \times L \tag{4} \]

The MDA concentration C (μmol/L) is calculated, and the MDA content C (μmol/g) is further calculated. Carbohydrates in plant tissues can interfere with the MDA-TBA response. To eliminate this interference, the following formula can be used to eliminate the error caused by sucrose.

\[ C/ \text{μmol/L} = 6.45(A_{532} - A_{600}) - 0.56 A_{450} \tag{5} \]

Take 2g of *peperomia tetraphylla* leaf (or stem) and wash, dry with filter paper, add 5ml of 5% trichloroacetic acid (TCA), grind in an ice bath, and centrifuge the resulting homogenate at 3000r/min for 10min.

Take 2ml of the supernatant, add 2ml of 0.67% TBA, mix and boil on a water bath at 100°C for 10 minutes, cool and centrifuge.

Measure the supernatant according to formula \(\text{(4)}\) respectively by using UV-2550 ultraviolet spectrophotometer at 450nm, 532nm and 600nm absorbance value, calculate the MDA concentration according to formula \(\text{(5)}\), and then calculate the MDA content [11].

3. Results

3.1. Changes in the fresh weight of *peperomia tetraphylla*

Select the *peperomia tetraphylla* that grows vigorously after domestication and culture, select 7 groups, 3 plants in each group (parallel test), weigh them separately, and record the data (see Table 3.1). After 7
...days, take the *peperomia tetraphylla* out of the cultured solution, wipe off the excess water, weigh them again, and record the data (see Table 1).

### Table 1. Changes in the fresh weight of *peperomia tetraphylla* after 7 days

| Concentration of phenol solution (mg/L) | weight of *peperomia tetraphylla* in Day1 (g) | weight of *peperomia tetraphylla* in the Day7 (g) | Difference (g) |
|----------------------------------------|-----------------------------------------------|-----------------------------------------------|----------------|
| 0                                      | 35.0                                          | 35.7                                          | 0.7            |
| 20                                     | 28.9                                          | 29.4                                          | 0.5            |
| 40                                     | 30.0                                          | 30.4                                          | 0.4            |
| 60                                     | 33.8                                          | 34.1                                          | 0.3            |
| 80                                     | 35.0                                          | 35.4                                          | 0.4            |
| 100                                    | 32.1                                          | 32.3                                          | 0.2            |
| 200                                    | 34.4                                          | 34.6                                          | 0.2            |

It can be seen from Table 1 that in the absence of phenol, *peperomia tetraphylla* can grow normally in water and grow well. When the water contains a small amount of phenol, it has already begun to affect the normal growth of *peperomia tetraphylla*. As the concentration of phenol increases, the impact on the growth of *peperomia tetraphylla* is also increasing. The study found that cultured at 80mg/L, 100mg/L, and 200mg/L concentrations which growth of *peperomia tetraphylla* in phenol solution slowed down significantly. It shows that phenol has an influence on the morphology of *peperomia tetraphylla*, and as the concentration of phenol increases, the influence of *peperomia tetraphylla* increases.

### 3.2. Changes of chlorophyll content in *peperomia tetraphylla* leaves and stems

Draw a graph with the average value of the chlorophyll content of leaves in different concentrations of phenol solution, as shown in Figure 1. As shown in Figure 1, *peperomia tetraphylla* grown in different concentrations of phenol solution is under different stress conditions. When the phenol concentration is 20-60mg/L, the concentration of chlorophyll a and b will be significantly reduced; when the phenol concentration is 80-100mg/L, the chlorophyll a, instead, the concentration of b increased, and the chlorophyll content was highest when the phenol concentration was 100 mg/L (the chlorophyll concentration at this concentration was higher than that of *peperomia tetraphylla* cultivated in clean water). It shows that the chlorophyll content in *peperomia tetraphylla* leaves can be reduced when there is a lower concentration of phenol, with the increase of phenol concentration, the chlorophyll content in *peperomia tetraphylla* leaves increases first and then decreases, reaching the highest value of chlorophyll content at 100mg/L.

![Figure 1. Changes of average value of the chlorophyll content of *peperomia tetraphylla* leaves under different concentrations of phenol solution](image-url)
3.3. Changes of MDA Content in *peperomia tetraphylla* leaves and stems

After 7 days, the content of MDA in the stems and leaves of *peperomia tetraphylla* was measured, and the absorbance values were measured at 450nm, 534nm and 600nm with UV-2550 ultraviolet spectrophotometer, and the MDA content was calculated according to the formula.

Draw a graph with the average value of the MDA content of leaves and stems in phenol solutions of different concentrations, as shown in Figure 2. It can be seen from Figure 2 that the stress conditions of *peperomia tetraphylla* grown in different concentrations of phenol are different. For the leaves of *peperomia tetraphylla*, when the phenol concentration is 60 or 200 mg/L, the content of MDA decreases; under other concentration conditions, the content of MDA increased. The content of MDA was the highest at 100mg/L which indicating that the membrane peroxidation of *peperomia tetraphylla* stems was enhanced at this concentration. For the stems of *peperomia tetraphylla*, when the phenol concentration is 20, 40, 100 mg/L, the MDA content decreases; under other concentration conditions, the MDA content increases, and at 200 mg/L, the MDA content The highest aldehyde content indicates that the membrane peroxidation of *peperomia tetraphylla* stems is enhanced at this concentration. Generally speaking, the content of MDA in *peperomia tetraphylla* stems is higher than that in leaves.

![Figure 2. Changes of average value of the MDA content of *peperomia tetraphylla* leaves and stems in different concentrations of phenol solution](image)

4. Conclusions

Through research on the morphological indicators of green plant *peperomia tetraphylla* under different concentrations of phenol, it is found that phenol affects the growth of *peperomia tetraphylla*. The higher the phenol concentration, the slower the growth of *peperomia tetraphylla*.

From the chlorophyll determination test in *peperomia tetraphylla* leaves, it can be seen that the chlorophyll content in *peperomia tetraphylla* leaves can be reduced when there is a lower concentration of phenol. As the phenol concentration increases, the chlorophyll content in *peperomia tetraphylla* leaves increases instead.

According to the measurement test of MDA content in *peperomia tetraphylla* leaves, when the phenol concentration is 100mg/L, the MDA content in *peperomia tetraphylla* leaves is the highest, and its membrane quality is strong.

From the determination test of MDA content in *peperomia tetraphylla* stems, it can be seen that when the phenol concentration is 200mg/L, the MDA content in *peperomia tetraphylla* stems is the highest, and its membrane quality is strong. Generally speaking, as the concentration of phenol increases, the content of MDA in *peperomia tetraphylla*'s body increases. When the phenol concentration is 80mg/L, the root system of *peperomia tetraphylla* begins to fall off which indicating that high concentration of
phenol damages the root system of *peperomia tetraphylla* seriously. In all the damage to stems and leaves is slight.

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