Phytoremediation of Waste Water Containing Phenol by *Salix Matsudana* Seedlings and their Physiological Response

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Abstract: In order to study the tolerance and removal capability of *Salix matsudana* to phenol wastewater, we determined the effects of different concentrations of phenol on the photosynthesis, chlorophyll fluorescence parameters and enzyme activities of *S. matsudana* cut seedlings and their purification effects to phenol using hydroponics. The results show that wastewater containing 30 mg L^{-1} and 60 mg L^{-1} phenol increased P_n, G_s and T_r of *S. matsudana*, however, the maximum photochemical efficiency (F_v/F_m) of PSII changed little. When the concentration of phenol increased to 90 mg L^{-1}-180 mg L^{-1}, P_n and F_v/F_m of *S. matsudana* were significantly lower than those in the control group, while the C_i increased significantly. The 30 mgL^{-1}-150 mgL^{-1} of phenol can increase the SOD and POD activities in leaves and roots of *S. matsudana* and the 180 mgL^{-1} of phenol decreased their activities. The percent removal of phenol decreased with increasing concentration of phenol ranging from 87% to 98% in 10 days. In conclusion, *S. matsudana* can be used to purify waste water containing phenol in concentration less than 150 mg L^{-1}.

Keywords: *Salix matsudana,* Phenol, Phytoremediation, Photosynthesis, Enzyme activity.

INTRODUCTION

At present, the water crisis and water pollution problems have aroused great attention from all countries. Wastewater containing phenol is widely existence and harmful. Wastewater containing phenolic compounds mainly from coking plants, gas plants, petrochemical plants and other industrial sectors, as well as synthetic dyes, organic pesticides and phenolic resin and other production processes [1]. In the coal gasification production process of waste water, the phenol concentration of up to 4000-6800mg /L. Car exhaust and cigarette smoke also have trace amounts of phenol. In addition to industrial waste water, feces and nitrogen-containing organic compounds also produce a small amount of phenolic compounds during the decomposition process. Therefore, the large amount of fecal effluent discharged from the city is also an important source of phenol pollutants in the water [2]. The human body intake of a certain amount of phenol will appear acute poisoning symptoms, long-term consumption of water contaminated with phenol can cause itching, dizziness, anemia and nervous system disorders [3]. The US Environmental Protection Agency has classified phenol as a priority pollutant [4]. Therefore, the study of the phenol pollution on our living environment and its removal method are of great significance.

Phytoremediation is a cost effective technology, and it takes advantage of the fact that a living plant can be compared to a solar driven pump, which can extract and concentrate particular elements from the environment. It entails the use of plants for uptake, sequestration, detoxification, or volatilization of inorganic and organic pollutants from soils, water, sediments, and possibly air [5]. There are already some reports of phytoremediation on phenol purification. Jha studied the repair of phenol by sunflower. The results showed that the sunflower root could purify 100 mg / L of phenol after 144 h of adaptation, and pointed out that catechol is the metabolite in the process of phenol removal [6]. Sosa studied the tolerability of tobacco under 100 mg / L phenol treatment and found that the antioxidant enzymes in plants play an important role in the fight against phenol stress, and the transgenic species are shorter than the time required for the activation of the enzyme activity in the wild species, showing a higher resistance [7]. *Salix matsudana* is one of the main tree species planted near water body. They are planted widely in China and has abundant resources, and it has great potential in terms of restoration of contaminated water or soil environment. At present, *Salix matsudana* has been mainly used in the remediation of petroleum hydrocarbons and polycyclic aromatic hydrocarbons (PAH) pollution and 2,4-dichlorophenol [8, 9]. Whereas, the effect of *S. matsudana* on the remediation of phenol in polluted water and its physiological response was not reported yet. Hence, the purpose of this investigation is to explore the tolerance and removal ability of *S. matsudana* to wastewater containing phenol.

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E-ISSN: 2311-858X/20

1. MATERIALS AND METHODS

1.1. Materials and Experimental Design

Branches of *Salix matsudana* trees, which are street trees of Donghu Road, Tai'an, Shandong, China, were cut from the middle of the tree crown. The branches were cut to 20 cm in length, and then put into buckets filled with tap water for rooting. Those cuttings in buckets were cultured in tap water in a plastic greenhouse located in Forestry Experimental Station of Shandong Agricultural University, Tai'an, Shandong, China. When the cuttings have lot of roots, then one seedling was transferred to a 500 mL conical beakers containing 400 mL half strength Hoagland nutrient solution. After their branches, leaves and roots developed well, the seedlings in similar growth status were taken for stress treatment.

One seedling was transferred to one conical beaker containing 400 mL phenol wastewater of concentration 0, 30, 60, 90, 120, 150 and 180 mg L^{-1} respectively, 4 replicate per treatment. From the day of treatment, observe the growth situation of the seedlings. After six days of treatment, the photosynthetic parameters and fluorescence parameters of the leaves were measured. After seven days of treatment, the enzyme activity was measured. After 10 days of treatment, the concentration of phenol was determined.

1.2. Research Methods

1.2.1. Determination of Photosynthetic Physiological Parameters

Two mature leaves of each plant in the experiment were selected for the determination of photosynthetic parameters by a portable photosynthesis system (CIRAS-2, PP SYSTEM, USA). The instrument parameters was set according to Xie H *et al.* [10]. Some parameters measured are net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), intercellular carbon dioxide concentration (Ci). The water use efficiency (WUE) and stomatal limitation (Ls) were calculated by using the formula: WUE = Pn / Tr, Ls = 1-Ci / Ca.

1.2.2. Determination of Fluorescence Parameters

After activation for 20 minutes in natural light, the maximum photochemical efficiency of plant leaves of different treatments was determined with a pulse amplitude-modulated chlorophyll FMS-2 fluorometer (FMS-2, Hansatech, Britain).

1.2.3. Determination of SOD and POD in Roots and Leaves

The roots and leaves of each plant were collected for enzyme activity. Peroxidase (POD) activity was measured by guaiacol method according to Liu Peng *et al.* [11]. Super oxide dismutase (SOD) activity was determined by nitrogen blue tetrazolium (NBT) photoreduction according to Liu Peng *et al.* [12].

1.2.4. Determination Concentration of Phenol

Residual phenol in cultural solution was measured by a colorimetric assay method [11]. The solution in the conical flask was sampled and then centrifuged in a high-speed refrigerated centrifuge. After centrifugation, 5 mL of supernatants was taken, 0.05 mL buffer solution was added, mixed, and then 0.1 mL of 4-aminoantipyrine solution was added, plus 0.1mL potassium ferricyanide solution, fully mixed, placed 10min. The absorbance of the reaction solution was determined at 510 nm. Calculate the concentration according to the standard curve.

1.3. Data Analysis

SPSS 17.0 and Excel 2010 software were used for data analysis. The mean number was analyzed by one-way ANOVA and differences were statistically significant with DUNCAN. The phenol purification efficient is calculated using the formula: Percent removal (%) = (initial phenol concentration - final phenol concentration) / initial phenol concentration×100.

2. RESULTS AND ANALYSIS

2.1. Physiological Response and Purification Effect of *Salix Matsudana* on Phenol Waste Water

2.1.1. Effects of Phenol Stress on Photosynthetic Parameters of Salix Matsudana

Different lowercase letters indicate means are significantly different from each other ($p<0.05$).

It can be seen from Figure **1** that the Pn of *Salix matsudana* increased first and then decreased with the increase of the concentration of phenol. When the phenol concentrations were between 30 and 60 mg / L, the Pn of the leaves was promoted. When the concentration of phenol was 90mg / L, the Pn began to decrease, and the Pn decreased with the increase of phenol concentration. This indicates that low concentration of phenol promotes the photosynthesis of *Salix matsudana* while high concentration of phenol inhibits the photosynthesis of *Salix matsudana*. With the increase of phenol concentration, the changes of

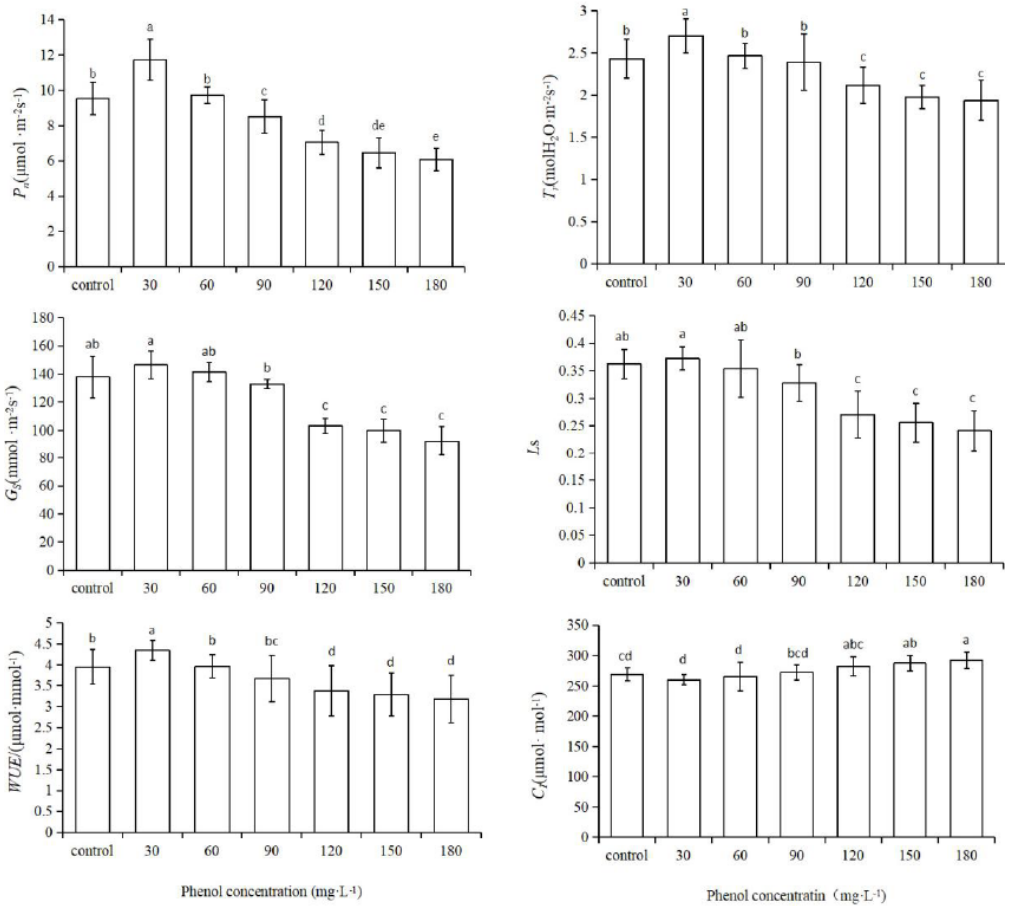

Figure 1: Photosynthetic parameters (means±SE) of *S. matsudana* under phenol stress.

Gs, Tr, WUE and Ls were the same as those of Pn, which showed the trend of increasing first and then decreasing. While the Ci showed the opposite trend, when the phenol concentration are 30, 60 mg / L, the Ci decreased; When the concentrations of phenol increased from 90 mg/L to 180 mg/L, the Ci showed a increasing trend.

2.1.2. Effects of Phenol Stress on Fluorescence Parameters of Salix Matsudana

The PSII maximum photochemical efficiency (Fv / Fm) of *Salix matsudana* decreased with the increase of the phenol concentration (Figure **2**). When the concentration of phenol was higher than 60mg / L, the Fv / Fm decreased rapidly and was different significantly to the control.

Different lowercase letters indicate means that are significantly differences from each other (P<0.05).

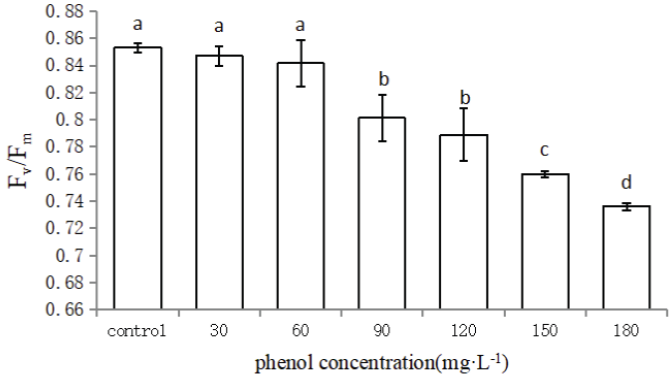

Figure 2: Fluorescence parameters (means ± SE) of *S. matsudana* under phenol stress.

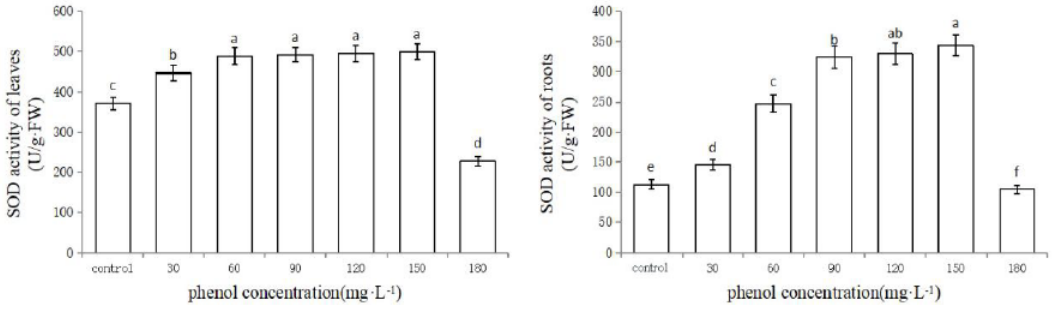

Figure 3: Effects of phenol stress on SOD activity of *S. matsudana* (means±SE).

2.1.3. Effects of Phenol Stress on the Enzyme Activity of Salix Matsudana

2.1.3.1. Effects of phenol stress on superoxide dismutase (SOD) activity of *Salix matsudana*

The SOD of *Salix matsudana* increased first and then decreased under phenol stress (Figure **3**). When the concentration of phenol was lower than 180 mg/L, the total activity of SOD in roots and leaves of *Salix matsudana* increased. When the concentration of phenol was 180mg / L, the total activity of SOD in roots and leaves of *Salix matsudana* decreased, and smaller than the control group.

Different lowercase letters indicate means that are significantly differences from each other (P<0.05).

2.1.3.2. Effects of phenol stress on the peroxidase activity of *Salix matsudana*

The effect of phenol stress on the activity of peroxidase (POD) of *S. matsudana* was similar to SOD (Figure **4**). The POD activity in the roots and leaves of *Salix matsudana* increased when phenol concentration increased from 30 to 150 mg/L. The POD activity was decreased under 180 mg/L phenol stress.

Different lowercase letters indicate means that are significantly differences from each other (P<0.05).

2.1.4. Removal Phenol by Salix Matsudana

From Table **1**, it can be seen that the percent removal of phenol by *S. matsudana* decreased with the increase of phenol concentration. When the phenol concentration was 30mg / L, the percent of removal was the highest, reaching 98.56%. When the phenol concentration was 180mg / L, the percent of removal was the lowest, reaching 87.03%. The average of the percent of removal was 89.83% in 10 days.

Table 1: Percent of Phenol Removal by *Salix Matsudana*

Initial Concentration Mg L^{-1}	Final Concentration Mg L^{-1}	Percent of Removal %
0	0	-
30	0.431±1.6e	98.564±5.6c
60	5.934±1.2d	90.110±2.0a
90	10.308±1.3c	88.546±1.5b
120	14.802±2.0b	87.665±1.7b
150	19.396±2.9a	87.073±2.0b
180	25.144±3.3a	87.031±1.8b

3. DISCUSSION

Plant photosynthesis is the vital process of energy conversion and organic material accumulation in plant production process. The photosynthesis is affected by

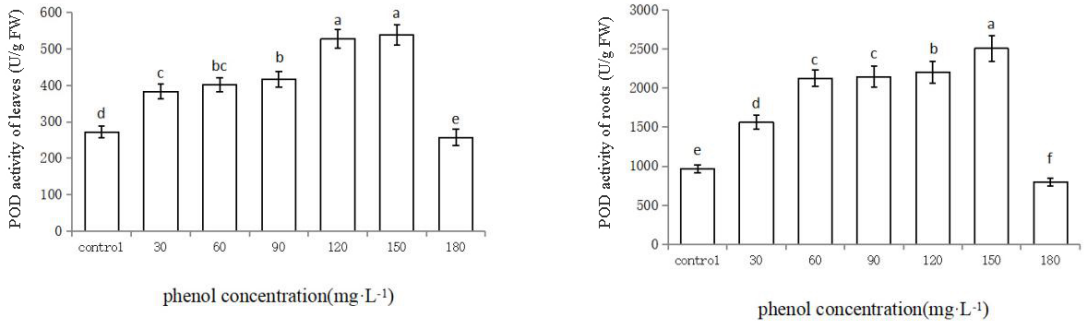

Figure 4: Effects of phenol stress on POD activity of *Salix matsudana* (means ± SE).

various environmental factors. Zhang Z *et al.* [13] found that low temperature, low light and salt stress could reduce Pn of pepper. The factors that usually affect plant photosynthesis can be divided into stomatal factors and non-stomatal factors. The former refers to water stress caused by decreased Gs, CO_2 into the leaves blocked so that photosynthesis decreased, the latter refers to the decrease in photosynthetic activity of mesophyll cells [14]. In this study (Figure **1**), the Pn of *S. matsudana* was higher than that of the control group when the concentration of phenol was 30-60 mg / L, and its water use efficiency was not significantly different from that of the control group, suggesting that low concentration of phenol could promote the physiological activities of *S. matsudana*. When the concentration of phenol reached or exceeded 90mg / L, the Pn and Gs of leaves decreased obviously, while the Ci increased. According to studies by Farquhar and Sharkey [15], this situation indicate that the main limiting factor for photosynthesis under high phenol concentration is non-stomatal factors, the photosynthetic structure of the *Salix matsudana* was damaged.

Chlorophyll fluorescence parameters in the determination of plant photosynthesis in the process of light system on the absorption of light energy, transmission, dissipation, and distribution has a unique role. Fv / Fm is the maximum photochemical quantum yield of PSII, which reflects the light energy conversion efficiency of PSII reaction center [16]. When the concentration of phenol was more than 60mg / L, the difference between Fv / Fm and control was significant (Figure **2**), indicating that the photosynthesis of the *S. matsudana* leaves was light-suppressed and the system of PSII has been destroyed.

SOD is an antioxidative enzyme with a crucial role in removing reactive oxygen species (ROS) in the organism. PODs are among the enzymes with a potential role in the detoxification of a variety of xenobiotics [17]. When the phenol concentration is between 30 and 150mg / L, the activity of SOD and POD in the leaves and roots of S. matsudana increases (Figures **3** and **4**), hence, the cells are protected from ROS injury. When the phenol concentration is 180 mg / L, the SOD and POD activities decreased, indicating that SOD and POD activities can not maintain a high level, and thus accumulation of ROS will cause damage to the cell membrane of plants. S. matsudana can endure 150 mg / L phenol , so it was suggested that S. matsudana can be used to phytoremediation of wastewater containing in concentration less than 150 mg / L.

The contaminant residues in the water can visually show the enrichment and removal of pollutants by plants, and the ability of the plant to degrade contaminants can be inferred by the presence of contaminants in the plant. The purification of phenol by *S. matsudana* decreased with the increase of phenol concentration. The average percent of phenol removal by *S. matsudana* is 89.8% in 10 days.

In conclusion, low concentration of phenol stress (<60mg / L) could promote the normal growth of *Salix matsudana*. When the concentration of phenol increased gradually, the photosynthetic rate of *S. matsudana* decreased, which affected the normal growth of *S. matsudana*. *S. matsudana* can be used to purify wastewater containing phenol in concentration less than 150 mg / L.

ACKNOWLEDGMENTS

This research was financially supported by Natural Science Foundation of Shandong Province as “The mechanism of purifying dye containing wastewater by phytoremediation” (Approved No.ZR2015CM021).

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Received on 24-08-2020 Accepted on 09-10-2020 Published on 30-10-2020

DOI: https://doi.org/10.12974/2311-858X.2020.08.6