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Original Research

Tolerance and Phytoremediation Capacity of *Salix matsudana* to Aniline Wastewater

Chaofan Sun[1], Huimin Zhou[1], Yujia Jiang[1], Yuanli Zhang[1],
Guilong Fu[1], Huicheng Xie[1,2*]

[1]Key Laboratory of State Forestry Administration for Silviculture of the Lower Yellow River, China
[2]Forestry College of Shandong Agricultural University, Taian, People's Republic of China

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Abstract

In order to explore the feasibility to use *Salix matsudana* in the phytoremediation of aniline contaminated wastewater, hydroponic experiment was conducted to study the effects of aniline stress on photosynthetic, chlorophyll fluorescence parameters and its removal by *S. matsudana*. It was found that the percent removal of aniline by *S. matsudana* was 78.25% on average. With the increase of aniline concentration, biomass, ΦPSII (Effective quantum yield of PSII photochemistry), Pn (Net photosynthetic rate), Tr (Transpiration rate), Gs (Stomatal conductance), Ls (Stomatal limitation), WUE (Water use efficiency), Fm (Maximal fluorescence), qP (photochemical quenching coefficient) and Fv/Fm (Maximal quantum yield of PSII photochemistry) of *S. matsudana* decreased significantly compared with the control group, while Ci (Intercellular CO_2 concentration), ETR (Electron transport rate) increased significantly, and NPQ (Non-photochemical quenching coefficient) increased firstly and then decreased. It is concluded that aniline inhibited Pn of *S. matsudana* was mainly through non-stomatal factors. Chlorophyll fluorescence parameter change showed that aniline damaged PSII photosynthetic system of *S. matsudana*. It is suggested that *S. matsudana* should be used in phytoremediation aniline when the aniline concentration was lower than 220 $mg \cdot L^{-1}$.

Keywords: aniline stress, photosynthesis, phytoremediation, *Salix matsudana*

Introduction

Aniline ($C_6H_5NH_2$) is the most representative substance of aromatic amines, and is widely used in plastic, rubber, national defense and pharmaceutical. Meanwhile, unreasonable discharge of industrial wastewater causes environmental pollution. It not only achieves good toxicity, inhibiting the growth of aquatic animals and plants, but is a teratogenic, carcinogenic and mutagenic pollutant. So aniline has been incorporated into the "China Environmental Priority Pollutant Black List". With the development of industrial production, the domestic production of aniline is continuing to rise. As the demand for aniline, the amount of emissions has increased constantly [1-3]. Therefore, how to remove aniline efficiently, conveniently, economically and ecologically has become the focus of research.

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*e-mail: xiehc@sdau.edu.cn

Compared with the physical, chemical and microbial remediation methods commonly used to purify aniline in the environment, phytoremediation can not only remove pollutants, but promotes the recycling and reuse of nutrients in sewage, while also greens the land and improves regional climate promotes a virtuous cycle of the ecological environment. Plants are known to remediate organic pollution contaminants from wastewaters [4-6]. Liu [7] used *Agropyron cristatum (L.) Gaertn* to carry out phytoremediation experiments on soil contaminated with aniline, found that the roots of *Agropyron cristatum (L.) Gaertn* transfer to leaves and stems. *Salix babylonica* can remove 2, 4-DNP efficiently, the percent removal of 2, 4-DNP in 20 $mg·L^{-1}$ wastewater was 91.4% [6]. When the aniline wastewater concentration was 30 $mg·L^{-1}$, Wang and Wen [8] studied the remediation effects of six aquatic plants on wastewater, and found that the plant's remediation efficiency for aniline in water bodies was 50.7%-97.3%, among which waterweed had the best remediation effect. When the aniline wastewater concentration was 38 $mg·L^{-1}$, Li [9] studied the remediation effect of nine aquatic plants on wastewater, and found that the removal efficiency of dripping Guanyin on aniline was the highest, reaching 99.6%, and did have no impact on the normal growth of plants. The purpose of this study is to reveal the tolerance and removal mechanism of *S. matsudana* to aniline and provide a scientific basis for its use in phytoremediation of wastewater containing aniline.

S. matsudana grows quickly and has a strong tolerance to metal, large biomass and an expanded root network, which are considered to be one of the suitable materials for Cd enrichment. *S. matsudana* is increasingly being cultivated worldwide for phytoremediation [10]. Wang and Ye [11] found that the use of *S. matsudana* floating bed could repair heavy metal pollution and lightly eutrophication of water. Tang [12] found that *S. matsudana* has a higher tolerance to Cd and absorption than *Typha angustifolia* by comparing them. Therefore, the use of *S. matsudana* to repair aniline wastewater has a bright future.

Materials and methods

Experimental Design

In mid-March, healthy branches were cut from *S. matsudana* trees in East Lake Park, Tai'an, Shandong, China (117°15′E, 36°19′N). Branches were cut into 20 cm sections in length and then were placed in baskets filled with tap water in a polytunnel greenhouse located in a forestry experimental station. When they took roots, each seedling was transplanted into one 500 mL conical beakers containing 400 mL half strength Hoagland's hydroponic nutrient solution. Those conical flasks covered by black plastic bags to deter algal growth. After four weeks of cultivation with 50% Hoagland culture solution, robust plants with consistent growth status were selected for aniline stress treatment.

Aniline stress treatment concentration was 0, 50, 100, 200, 300 and 400 $mg·L^{-1}$ [13], respectively. Triplicates were set for each treatment. After 7 days of treatment in the natural environment, photosynthetic parameters, fluorescence parameters, spectrum parameters were determined. Then, the culture solution level in the triangle bottle was added to 400 mL with deionized water, shaken evenly, then 10 mL aliquot was taken to the centrifugal tube for aniline concentration measurement. Root of seedling was flushed with distilled water, then the seedlings were cut into leaves, stem and root. The fresh weight of each organ was evaluated.

Measuring Items and Methods

Photosynthetic Physiological Parameters

Photosynthetic parameters and chlorophyll fluorescence measurements were measured using a CIRAS-2 portable photosynthesis system (Systems PP, USA) and a pulse amplitude-modulated chlorophyll FMS-2 fluorometer (Hansatech, King's Lynn, UK) respectively. Procedures in detail were according to a pervious study [14]. WUE and stomatal limitation value Ls were calculated by formula respectively: WUE = Pn/Tr, Ls = 1 - Ci/Ca, where Ca is the concentration of air CO_2 [15-16]. According to the formula [17-18], we can calculate the following parameters: Fv/Fm = (Fm - Fo)/Fm; ΦPSII = (Fm' - Fs)/Fm'; qP = (Fm' - Fs)/(Fm' - Fo'); NPQ = (Fm - Fm')/Fm'.

Spectrum Parameters Measurement

A Unispec-SC (single channel) portable spectrometer produced by PP Systems (USA) was used to measure the spectral reflectance of three healthy *S. matsudana* leaves in the different treatments. Procedures of measurement and data processing in detail were according to a pervious study [19].

Determination Concentration of Aniline

Water samples were syringe filtered through 0.22 mm nylon filters for HPLC analysis. The aniline concentration was determined at 240 nm using Waters Acquity UPLC (Waters Corporation, USA) equipped with an Acquity UPLC BEH C18 column (1.7 µm/2.1 × 50 mm) and a UV detector. A mixture of methanol and H_2O (30:70 (v/v), pH3.0 adjusted with 0.1mol L^{-1} H_3PO_4) was invoked as the mobile phase at a flow rate of 0.2 $mL·min^{-1}$, and a 3µL injection volumes.

Measurement Biomass of S. matsudana

Firstly, samples of roots, stems and leaves of *S. matsudana* were washed with deionized water and then dried with absorbent paper after the experiment. Then they were cut off with pruning scissors and put into envelopes respectively. Finally, they were dried in an oven until constant weight. The water content was estimated and then the biomass of sunflower organs was calculated.

Data Analysis

We used SPSS 22.0 and Origin 2019 software to sort out the experimental data, analyzed the average by one-way ANOVA, and compared the difference by Duncan.

Results

Effects of Aniline Stress on Photosynthetic Parameters of *S. matsudana*

Fig. 1 described the photosynthetic parameters of *S. matsudana* leaves treated with different concentrations of aniline. With increasing concentrations of aniline, the Pn, Tr, Gs and Ls of *S. matsudana* declined. Pn even decreased significantly ($P<0.05$) when the concentration of aniline reached 100 mg·L^{-1}. When aniline concentration was 400 mg·L^{-1}, Pn, Tr, Gs, Ls were only 12.5%, 20%, 19.8%, 5% of the control group ($P<0.05$). The trend of Ci was contradictory to others, increased with the increase of aniline concentration. The result indicated that the main cause of Pn decrease was

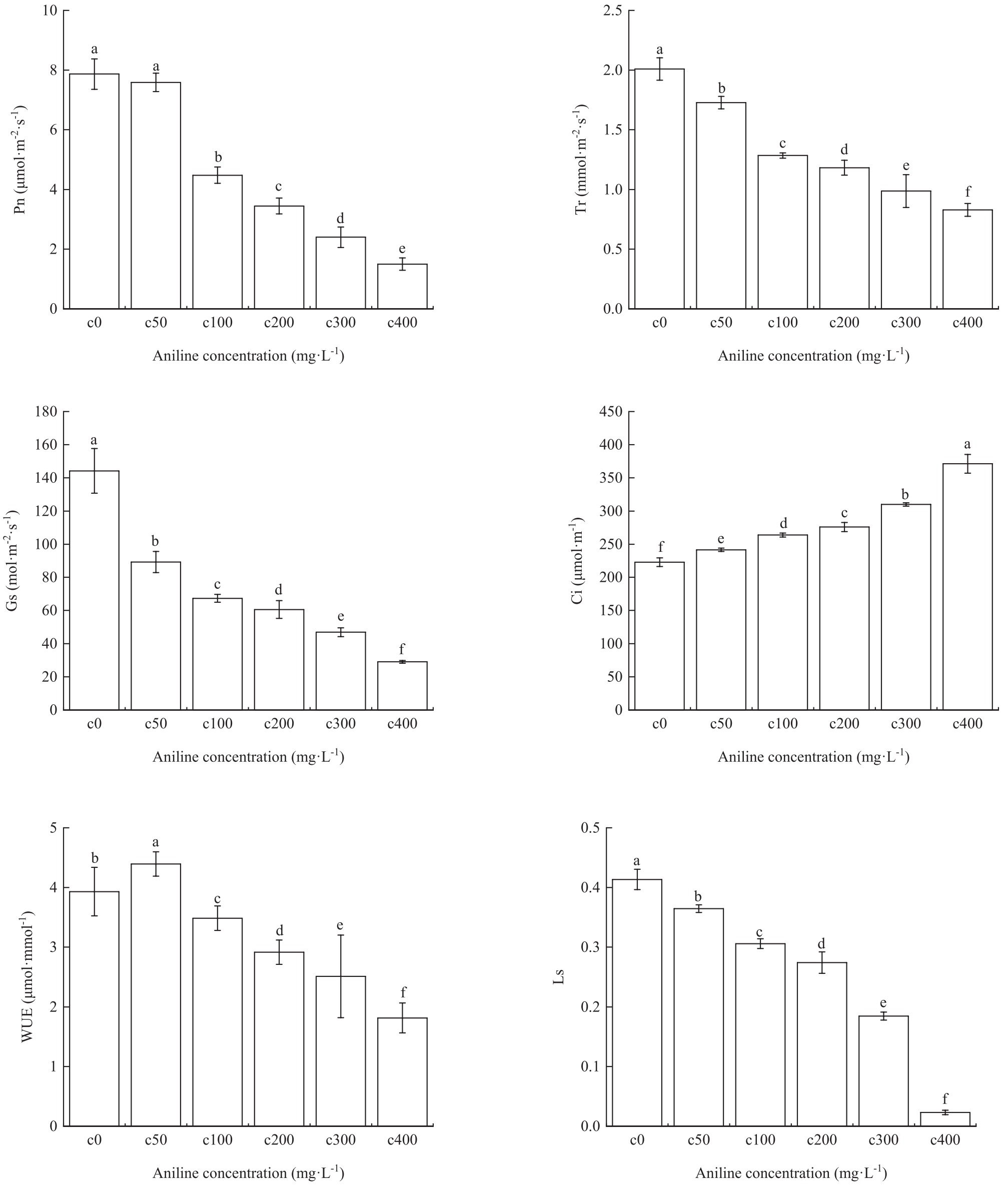

Fig. 1. Photosynthetic parameters of *S. matsudana* under aniline stress (means±SD).
Different lowercase letters mean significant difference among treatments at 0.05 level. Pn: Net photosynthetic rate; Tr:Transpiration rate; Gs: Stomatal conductance; Ci: Intercellular CO_2 concentration; WUE: Wateruse efficiency; Ls: Stomatal limitation.

changed from stomatal factor to non-stomatal factor. In addition, WUE increased first and then decreased.

Effects of Aniline Stress on Fluorescence Parameters of *S. matsudana*

Fig. 2 described the fluorescence parameters of *S. matsudana* leaves treated with different concentrations of aniline. The overall trend of Fo and ETR were increased by 18.66% and 108.90% compared with the control group, indicating that high aniline stress inhibited the PSII reaction. On the contrary, Fm, Fv/Fm, ΦPSII, Fv′/Fm′ and qP decreased by 25.75%, 15.07%, 71.55%, 43.27% and 44.21%. Even NPQ first increased and then decreased, that was, when the aniline concentration was 0-100 mg·L^{-1}, it increased

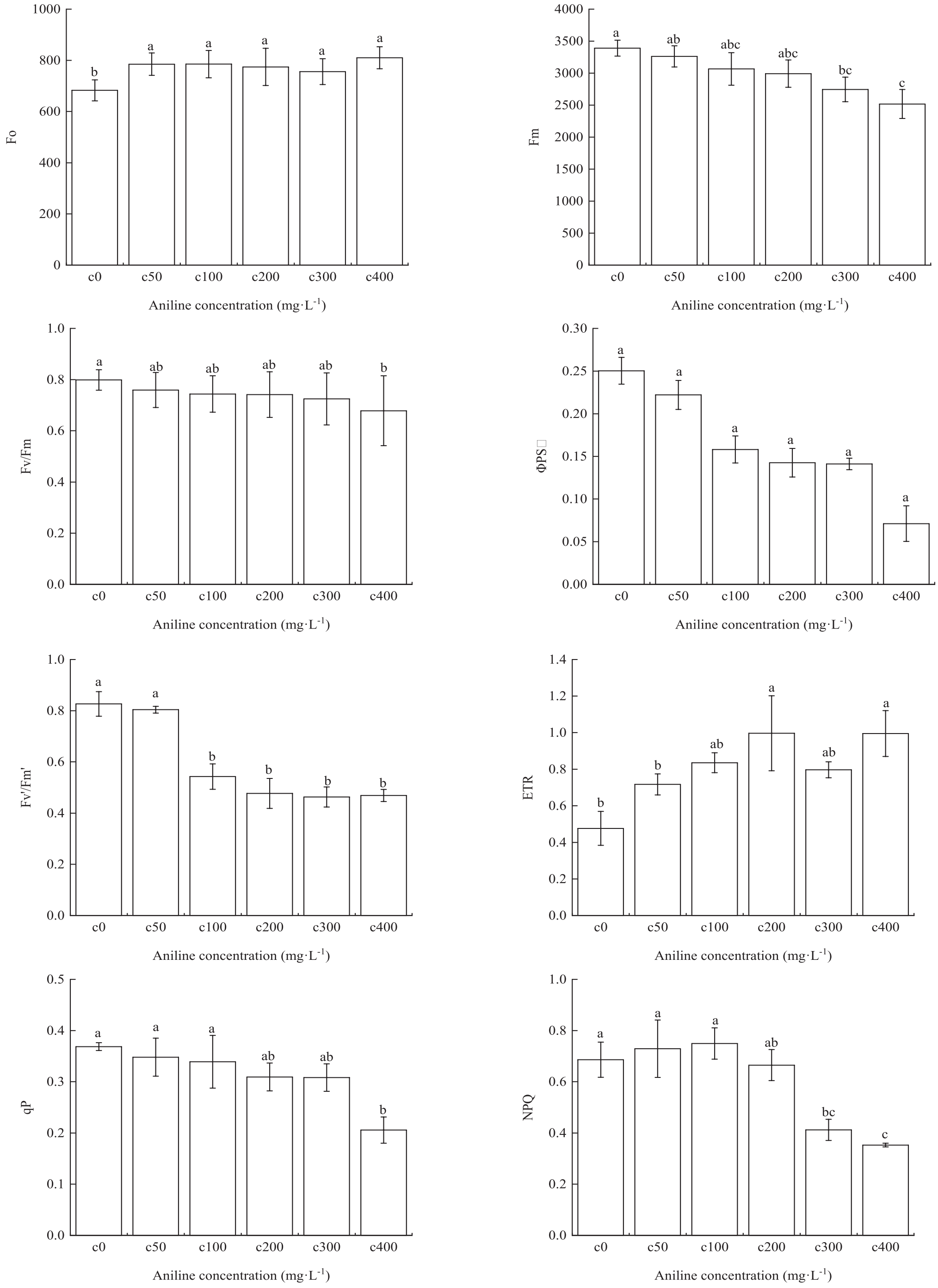

Fig. 2. Fluorescence parameters of *S.* matsudana under aniline stress (means±SD).

Different lowercase letters in the same column mean significant difference among treatments at 0.05 level. Fo: Minimal fluorescence; Fm: Maximal fluorescence; Fv/Fm: Maximal quantum yield of PSII photochemistry; ΦPSII: Effective quantum yield of PSII photochemistry; Fv′/Fm′: Maximum PSII aantenna conversion effciency; ETR: Electron transport rate; qP: Photochemical quenching coefficient; NPQ: Non-photochemical quenching.

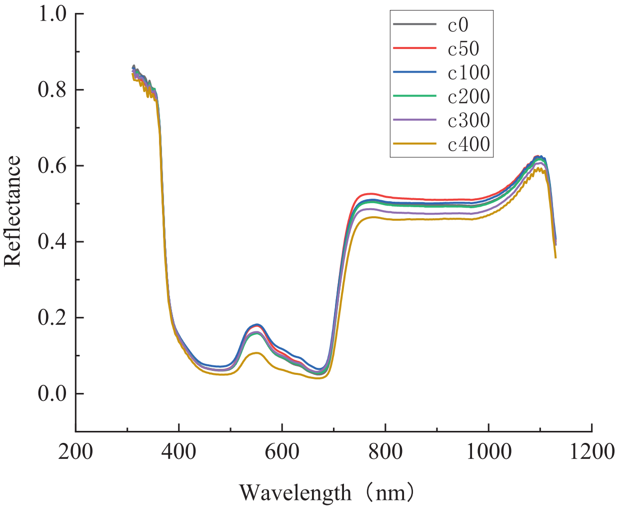

Fig. 3. Spectral reflectance curves of *Salix matsudana* seedlings treated with different aniline concentrations and light intensities.

with the increase of aniline concentration, and when the aniline concentration was 200-400 mg·L^{-1}, it decreased. The reduction of ΦPSII and Fv/Fm shows that PSII potential activity decline, reduced qP that PSII reduction reaction centers of activity [20], showed that aniline stress can inhibit or reduce chemical plants leaf PSII photochemical activity and light efficiency.

Effect of Aniline on the Reflectance Spectra

Fig. 3 showed that the spectral reflection curves of *S. matsudana* cuttings treated with different aniline. These curves had roughly the same trend. There were two low reflection regions at 500 nm and 680 nm, which is caused by the absorption of chlorophyll in the blue and red light bands for photosynthesis. At 550 nm reflection peak, different aniline the spectral reflectance of the *S. matsudana* cuttings treated was: c100> c50> c300> c200> c0> c400. At 300-400 nm and 1100 nm, the spectral reflectance of *S. matsudana* cuttings with high concentration of aniline treatment (c400) is more low than that in low concentration of aniline treatment (c50, c100, c200, c300). At 1100 nm reflection peak, different aniline the spectral reflectance of the *S. matsudana* cuttings treated was: c50> c0> c100> c200> c300> c400.

The plant spectral index reflects the internal pigment content of the plant, including chlorophyll and carotenoids. It can be seen from Table 1 that the difference in spectral index between *S. matsudana* cuttings treated with different anilines is significant: in addition to the normalized difference vegetation (NDVI) and the structure-independent pigment index (SIPI), the difference is not significant ($P<0.05$). When the concentration of aniline reached 400 mg·L^{-1}, the carotenoid reflectance index (CRI) of *S. matsudana* seedlings was the highest, up to 0.16, which was 4 times that of the control group, and there was a significant difference between control and other treatment groups ($P<0.05$). Under stress of 200 mg·L^{-1} aniline, the SIPI was 0.73, which was the lowest value in the treatment group and significantly different from other treatment groups ($P<0.05$). In a nutshell, high concentration aniline treatment will significantly reduce Chl normalized difference index (ChlNDI) and photochemical reflectance index (PRI) ($P<0.05$), but significantly increased the CRI ($P<0.05$).

Biomass and Purification Effect of *S. matsudana* on Aniline

With the increase of aniline treatment concentration, the biomass of *S. matsudana* decreased significantly (Table 2). When the aniline concentration was 200 mg·L^{-1}, the biomass of *S. matsudana* was significantly lower than that of control group ($P<0.05$). When the aniline concentration was 400 mg·L^{-1}, the biomass decreased by 77.8%, but the plants did not die, indicating that lethal concentration of aniline was not reached in the one-week culture period. With the increase of aniline concentration, the aniline removal rate of *S. matsudana* decreased first and then increased. When the aniline concentration was 100 mg·L^{-1}, the percent of removal was the highest, reaching 88.583%.

Table 1. Spectral index of *Salix matsudana* seedlings under different aniline concentrations (means±SD).

Treatment	Normalized difference vegetation, NDVI	Chl normalized difference index, ChlNDI	Carotenoid reflectance index, CRI	Structure-independent pigment index, SIPI	Photochemical reflectance index, PRI
c0	0.80±0.02	0.45±0.02^{a}	0.04±0.05^{b}	0.75±0.00^{ab}	0.05±0^{a}
c50	0.80±0.02	0.49±0.05^{a}	0.06±0.12^{ab}	0.75±0.00^{ab}	0.04±0.01^{ab}
c100	0.79±0.03	0.40±0.03^{a}	0.01±0.12^{b}	0.73±0.01^{c}	0.03±0.00^{ab}
c200	0.79±0.01	0.42±0.07^{a}	0.09±0.09^{ab}	0.74±0.01^{bc}	0.04±0.01^{ab}
c300	0.80±0.01	0.38±0.03a	0.04±0.03^{b}	0.76±0.01^{ab}	0.03±0.00^{ab}
c400	0.81±0.02	0.34±0.04b	0.16±0.15^{a}	0.77±0.02^{a}	0.02±0.01^{b}

Different lowercase letters mean significant difference among treatments at 0.05 level.

Table 2. Changes of the biomass of *S. matsudana* and its effect on the restoration of aniline (means±SD).

Aniline concentration(mg/L)		Removal rate	Biomass/(g)		
Before the test	After the test		Leaf	Stem	Root
0	0^{e}	——	$9.180±0.445^{a}$	$78.367±1.836^{a}$	$5.280±0.169^{a}$
50	$6.198±0.282^{de}$	$87.604±0.564^{b}$	$8.655±0.496^{b}$	$72.312±1.480^{b}$	$4.542±0.184^{ab}$
100	$11.417±0.547^{d}$	$88.583±0.547^{b}$	$7.193±0.391^{bc}$	$67.790±1.852^{bc}$	$3.994±0.278^{bc}$
200	$59.753±4.794^{c}$	$70.124±2.397^{d}$	$5.560±0.675^{c}$	$65.213±1.568^{c}$	$3.522±0.248^{cd}$
300	$90.517±4.231^{b}$	$69.828±1.410^{d}$	$4.468±0.750^{d}$	$58.826±1.716^{d}$	$3.096±0.258^{d}$
400	$99.458±2.999^{a}$	$75.136±0.750^{c}$	$2.122±0.457^{e}$	$52.824±1.215^{e}$	$2.052±0.278^{e}$

Different lowercase letters mean significant difference among treatments at 0.05 level.

Discussion

Photosynthetic Characteristics of *S. matsudana*

Photosynthesis provides material and energy for plant growth and is just an indispensable and important process for plants [17-18]. When the concentration of aniline increased gradually, Pn of *S. matsudana* decreased compared with the control group. It suggested that aniline at lower concentrations have an inhibitory effect on the normal physiological activities of *S. matsudana*, and the more seriously inhibitory effect produced with the increase of aniline concentration. The relationship between the concentration of aniline and the Pn was well simulated by a line regression model, $Y = -0.016316X + 7.401578$, $R^2 = 0.87$, $P<0.01$. Among them, Y is Pn and X are the aniline concentration. It is calculated that the concentration of aniline is 226.82 $mg·L^{-1}$ when the percentage of the photosynthetic rate is 50% of the control. Its is suggested that *S. matsudana* was only suitable for treating wastewater with aniline concentration lower than 200 $mg·L^{-1}$. In a previous study, when the 2, 4-dinitrophenol concentration reached 20 $mg·L^{-1}$, Pn was 41% of the control group, while in 25 $mg·L^{-1}$ and 30 $mg·L^{-1}$ solutions, all the *Salix babylonica* seedlings died [6]. This indicates that 2, 4-dinitrophenol is more toxic than aniline.

Ci is a very important index and basis for analyzing stomatal limitation and non-stomatal limitation of leaf photosynthesis [15-16]. Generally speaking, stomatal factor means that stomatal conductance restricts CO_2 entry into leaves and reduces photosynthesis, while non-stomatal factor indicates that photosynthetic activity of mesophyll cells is reduced [21]. The decrease of Pn, Gs and increased Ci showed that the main limiting factor of photosynthesis in *S. matsudana* under aniline stress was the non-stomatal factor [6]. When *S. matsudana* were subjected to non-stomatal restriction, which negatively affected photosynthesis of plant leaves [22]. Under external stress, chloroplasts expanded and the ultrastructure of photosynthetic organs was destroyed, which affected Pn. The results are similar to those of Li et al [19]. Shi et al [23] and Wang et al [24] reported the effect of chloroplast structure destruction on Pn in wheat and *Ginkgo biloba* respectively. For any living creature living on earth, there is usually no single stress, but a long period of exposure to multiple simultaneous threats [25]. So in field condition, the tolerance of plants to contaminant stress is more complicated.

Chlorophyll Fluorescence Parameters of *S. matsudana*

As one of the important indicators reflecting plant photosynthesis process, chlorophyll fluorescence parameters can be used to monitor plant photosynthesis rapidly and without damage from the perspectives of energy absorption, transfer and conversion, and is often used as a probe to indicate the degree of environmental stress [26]. Fo is the fluorescence level when all the PSII reaction centers are open. The destruction or reversible inactivation of the PSII reaction centers causes an increase of Fo [27-28]. Fv/Fm refers to the maximum photochemical efficiency of PSII, reflecting the original light energy conversion efficiency in the PSII reaction center. Fv/Fm is highly positively correlated with the growth state of plants, and it is an important parameter indicating the photochemical reaction status [29-30]. In this study, with the increase of aniline concentration, the Fo increased and the maximum fluorescence Fm decreased, which was consistent with the results of Li et al [19].

ΦPSII is a product of qP and can be used to assess the contribution of non-photochemical quenching to PSII changes and to indicate the extent to which the PSII center is open [31]. The higher the qP value is, the higher the electron transfer activity of PSII is. In this study, qP decreased with the increase of aniline concentration, suggesting that the decrease in the proportion of open centers and the decrease of non-photochemical quenching is the reasons for the higher ΦPSII in aniline-treated plants. NPQ is a proxy for the photoprotective heat dissipation process that regulates photosynthetic light harvest [32]. Plants can protect their photosynthetic apparatus from damage by increasing

NPQ [33]. Our results demonstrated that the increase aniline concentration triggers a significant increase in NPQ, highlighting the occurrence of photoprotection through the NPQ pathway. This increase in NPQ is related to the lutein cycle, which can promote the deepoxidation of ananthaxanthin into xanthin and zeaxanthin [34]. However, at a given light intensity, the increase of NPQ may be at the expense of Pn, thus weakening the carbon fixation ability [35].

Reflectance Spectra of *S. matsudana*

Spectral data are essential for determining quantitative and qualitative relationships between optical properties and plant composition [36]. This study showed that the spectral reflectance of leaves was substantially responsive to aniline stress. In the wavelength range of 400-1000 nm, the leaves of both control and treatment groups showed strong reflectance in the visible spectral region, with a strong peak reflectance at 550 nm. At 700-750 nm, the reflectivity increases rapidly. The increase rate slows down at 750-1000 nm. The result is a superior "reflective platform". When at 550 nm, the reflectance of *S. matsudana* showed a decreasing trend with the increase of aniline concentration. When at 750-1000 nm, c0> c50> c10 > c200> c300> c400. With the increase of aniline concentration, the reflectance of *S. matsudana* seedling leaves decreased gradually. In the infrared range, the exact reflection spectrum of leaves showed obvious changes. These results indicated that it is feasible to determine the effect of aniline on *S. matsudana* using infrared light differential diagnostic technology [19, 37].

Removal and Biomass of *S. matsudana*

Biomass change is the most intuitive manifestation of plants under stress [38]. Meanwhile, in many studies, biomass has been used to indicate the tolerance of plants to stress [39-41]. Plant biomass will decrease with the increasing degree of stress [41-42]. This studied showed that biomass of *S. matsudana* decreased with the increase of aniline concentration in water. When the aniline concentration was 100 mg·L^{-1}, the percent removal of aniline by *S. matsudana* was the highest, reaching 88.583%.

Conclusion

In conlusion, aniline stress could cause significant toxicity to *S. matsudana*. As the concentration of aniline increased, the Pn and biomass of *S. matsudana* decreased significantly. The inhibition of photosynthetic by aniline is attributable to non-stomatal factors. Furthermore, chlorophyll fluorescence parameters showed aniline damaged PSII photosynthetic system of *S. matsudan*a. Finally, *S. matsudana* had a strong ability to remove aniline from wastewater and it is suggested that *S. matsudana* should be used in phytoremediation aniline when the aniline concentration was lower than 220 mg·L^{-1}.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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