International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 12-02-2019; Accepted: 15-03-2019
www.botanyjournals.com
Volume 4; Issue 3; May 2019; Page No. 43-51

The effects of exogenous selenium on 2, 4 dichlorophenol induced physiological response in *Salix matsudana*

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Abstract
In order to determine the feasibility of using *Salix matsudana* to repair wastewater contaminated by 2, 4-dichlorophenol, we investigated the physiological response of *Salix matsudana* to 2, 4-dichlorophenol and the effects of exogenous selenium (Se) application on the tolerance of *Salix matsudana* to 2, 4-dichlorophenol stress. The clonal plants of *Salix matsudana* were cultured in Hoagland solution supplied with 2, 4-dichlorophenol (0, 5, 10, 12.5, 15 $mg \cdot L^{-1}$) and Se (2 $\mu mol \cdot L^{-1}$), separately or simultaneously. It was shown that the exposure to 2, 4-dichlorophenol led to significant changes in the photosynthetic physiological parameters of *Salix matsudana*: net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (E), chlorophyll content decreased, and superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activity increased first and then decreased. The addition of Se increased Pn, maximum photochemical calibration rate (Fv/Fm), chlorophyll content and decreased the coefficient of photochemical quenching (NPQ), SOD, CAT activities. But Se enhanced POD activities and decreased E and Gs at low concentration of 2, 4-dichlorophenol. The results imply that with the increase of the concentration of 2, 4-dichlorophenol, the toxicity will be strengthened, and the plant will make corresponding reaction mechanism, such as the increase of initial antioxidant enzyme activity, and the degree of disturbances caused by 2, 4-dichlorophenol could be partially ameliorated by Se supplementation. However, the effect of Se on the 2, 4-dichlorophenol-stressed plants depended on the concentration of Se and application of Se may be a risk factor in low concentration 2, 4-dichlorophenol.

Keywords: 2,4-dichlorophenol stress, photosynthesis, selenium addition, *Salix matsudana*

1. Introduction

Chlorophenols are widely used, highly toxic and seriously polluted substances. The toxicity of 2, 4-dichlorophenol is moderate. 2, 4-dichlorophenol is mainly used as pesticide and pharmaceutical intermediates. 2, 4-dichlorophenol has been listed as a priority pollutant by the U.S. Environmental Protection Agency (EPA) and the Chinese government [1]. It widely exists in the water environment of our country [2]. and its concentration in some water bodies can reach 0.1-1 mg/L [3]. This concentration can affect the human endocrine system [4]. There are many studies on phenol, but there are few studies on 2, 4-dichlorophenol, especially on plant toxicity and its removal by plants. So the study of plant tolerance to 2, 4-dichlorophenol is of great practical significance.

Physical, chemical and biological methods have been applied to remove 2, 4-dichlorophenol from industrial wastewater. Among the methods of purifying toxic substances in the environment, physical and chemical methods are easy to produce secondary pollution, have high cost and do not yield full purification. In addition, due to the inhibition of phenolic substances on microbial metabolism, the biodegradation of traditional activated sludge systems is usually slow [7]. Because there are much disadvantages of traditional pollutant removal technologies, more and more new technologies have emerged. For example, the use of enzymes, such as polyphenol oxidase (PPO) and peroxidase (POD), has attracted wide attention and many previous studies researched about it. Enzyme-based technologies have many advantages over the use of microbial cells [10]. The potential advantages of the enzymatic treatment as compared with conventional treatments include: application to recalcitrant materials, operation at high and low contaminant concentrations over a wide pH, temperature and salinity range, needs of biomass acclimatization and the easy control process among others [11]. However, the enzymatic treatment was not applied on an industrial scale, mainly because of the high cost of enzymatic treatment and the losses in enzymatic activity caused by adsorption of enzyme molecule son end-product polymers [8]. Finally, it has been found that plant materials can help to remove phenolic compounds from water, and peroxidase in plant tissues plays a detoxifying role [9]. Phytoremediation is a plant-based and cost-effective remediation method, which has attracted wide attention because of its low cost, easy operation and environmental friendliness. Algae are found to have phenol degrading activity [6]. But not all plants can accumulate heavy metals or organic pollutants because of their physiological differences. Even different species within the same species have different abilities to accumulate pollutants. So it is necessary to study the relationship between the selected plants and pollutants before phytoremediation. Although phytoremediation can tackle environmental problems in situ, its main disadvantage is that it requires long-term investment, because this process depends on the ability of plants to grow in an environment that is not suitable for normal plant growth. Previously, many researchers suggested that selenium exert a positive effect on crop growth and stress tolerance at low concentration [5].

Therefore, in order to improve this process, selenium was added to some extent to improve the growth and repair ability of plants in harsh environment.

Woody plants do not participate in the food chain. Compared with herbaceous plants, woody plants have high biomass, high transpiration rate and they are energy plants, so they have more application value. Willow is one of the afforestation tree species in China. It is deciduous and fast-growing, has developed roots, has strong adaptability and stress resistance, and is easy to reproduce. At present, willow is mainly used in the remediation of petroleum hydrocarbons and polycyclic aromatic hydrocarbons (PAH) pollution. However, there are few reports on the remediation of 2, 4-dichlorophenol in water by willow [12]. In this experiment, the growth status, photosynthetic parameters, fluorescence parameters, chlorophyll content and enzyme content of *Salix matsudana* exposed to 2, 4-dichlorophenol at different concentrations were observed, in order to study the feasibility of *Salix matsudana* as a phytoremediation material to remove 2, 4-dichlorophenol.

Although the effect mechanism of selenium on plants has not been fully revealed, whether selenium is a necessary element for plant growth and development has not yet been determined. However, it is generally believed that high concentration of selenium has toxic effects on plants, but low concentration of selenium can obviously promote plant development and strengthen the resistance to stress [13]. When plants are treated with stress, they produce active oxygen components, which are used as signals to produce antioxidant substances, such as ascorbic acid, tocopherol and various antioxidant enzymes, such as peroxidase, superoxide dismutase, glutathione peroxidase and ascorbic acid peroxidase. These enzymes in selenium-added plants will be enhanced [14]. In addition, there are many reports that selenium can inhibit the active oxygen components produced by ultraviolet radiation, drought, low temperature and high temperature [15]. Selenium in plants also plays a role in the repair of cell membrane structure damage in stressed plants. Filek *et al*. found that 2 micromol selenite could promote the reorganization of mechanism structure and thylakoid body and enhance the fluidity of its membrane, and could repair the damage of chloroplast membrane structure caused by Cd. Secondly, selenium in plants can alleviate the toxic side effects of heavy metals in plants [16]. In order to study whether selenium is beneficial to plant growth and stress resistance, selenium supplementation group was set up and compared with control group.

2. Material and Methods

2.1 Experimental Design

Salix matsudana was used as experimental material in hydroponic experiment. Branches of *Salix matsudana* trees, which were planted at the shores of East Lake Park, Tai'an, Shandong, China, were cut from the middle of the tree crown. The branches were cut to the size of 20 cm in length, and then inserted into an Erlenmeyer flask of 250 ml filled with tap water for two weeks. Then they were cultured in half strength Hoagland nutrient solution. After its branches, leaves and roots developed well, the seedlings in similar growth status were taken for stress treatment.

They were divided into two groups, each with five treatments. The concentration of 2, 4-dichlorophenol in one group was 0, 5, 10, 12.5 and 15 $mg \cdot L^{-1}$, respectively. The concentration of 2, 4-dichlorophenol in the other group was the same as that in the previous group, but 2 $\mu mol \cdot L^{-1}$ selenium was added at the same time. Each treatment has three replicates. After three days of stress treatment, the treatment solution was reconfigured and replaced. On the seventh day after treatment, photosynthetic parameters and fluorescence parameters were measured. Leaves were collected and stored in liquid nitrogen for the determination of activities of CAT, SOD, POD and chlorophyll content.

2.2 Items and Methods of Measurement

2.2.1 Measurement of photosynthetic physiological parameters

The CIRAS-2 photosynthesis system produced by PPS Company in Britain was used to measure the photosynthetic response under different stresses by selecting three robust mature leaves from each experimental plant. The measurement time was between 8:30 and 11:00 in sunny day. The same leaf labeled on each plant was measured. Each leaf was recorded three times and the average value was taken for analysis. Atmospheric carbon dioxide concentration (Cr), intercellular carbon dioxide concentration (Ci), transpiration rate (E), stomatal conductance (Gs) and net photosynthetic rate (Pn) were measured.

2.2.2 Chlorophyll fluorescence parameter determination

After the plants were activated under sunlight for a period of time, the pulse amplitude-modulated chlorophyll FMS-2 fluorometer (Hansatech, King's Lynn, UK) was used to determine the minimum fluorescence (Fo'), maximum fluorescence (Fm'), actual fluorescence yield (Fs) and maximum photochemical efficiency (Fv/Fm) of *Salix matsudana* leaves treated with or without selenium at different concentration gradients of 2, 4-dichlorophenol. Those measurements were carried out between 8:30 and 11:00 h. The initial fluorescence (Fo) and maximum fluorescence (Fm) were determined after 30 min dark adaptation. The maximal

quantum yield of PSII photochemistry, actual photochemistry efficiency, photochemistry quenching coefficient and non-photochemistry quenching coefficient of PS II were calculated according to the formula. The calculation formulas are as follows:

$Fv/Fm = (Fm-Fo)/Fm$ [17];

$PS\ II = (Fm'-Fs)/Fm'$ [17];

$\Phi_{PS\ II} = (Fm'-Fs)/(Fm'-Fo')$ [18];

$NPQ = (Fm-Fm')/Fm'$ [18].

2.2.3 Determination of Enzyme Activity

Nitroblue tetrazolium method (NBT) was used to determine superoxide dismutase (SOD) activity [19]. One unit of SOD activity was defined as the amount of enzyme that caused 50% inhibition of photochemical reduction of NBT, expressed in U/g. Guaiacol chromogenic method was used to determine peroxidase (POD) activity [20], 470 nm per minute change in the optical density (D470nm) under 0.10 was defined as the activity of a unit (U). Catalase activity (CAT) was performed referring to Wang Yongjun's Classical Method.

2.2.4 Determination of chlorophyll content

A certain amount of fresh leaves was weighted by cutting a certain amount of mesophyll while avoiding veins and the quantity of specific sample was recorded. Subsequently, the sample was put into centrifugal tube and leached with 80% acetone solution for 5 ml. Leaching chlorophyll in a refrigerator at 4 ℃ for about 24 hours under dark conditions, shaking every 8 hours to ensure the full leaching of chlorophyll, then the samples were centrifuged at a speed of 3000r·min^{-1} for 10 min (medical centrifuge TGL-185M). The absorbance (OD) of supernatant at 470, 646 and 663 nm was determined by UV-1600 ultraviolet spectrophotometer (MAPADA) with 80% acetone solution added to a colorimetric dish. The absorbance values obtained were substituted into the formula to calculate the chlorophyll a (Chla) and chlorophyll b (Chlb) concentrations (mg·g^{-1}) in leaves. Chla content = 12.21 * OD_{663}-2.81 * OD_{646}: Chlb content = 20.13 * OD_{646}-5.03 * OD_{663}. The sum of Chla and Chlb is the total chlorophyll concentration. According to the following formula, the total chlorophyll content per unit fresh leaf mass was calculated. Total chlorophyll content (mg·L^{-1}) = [total chlorophyll concentration (mg·L^{-1})* extraction volume (L)* dilution multiple]/fresh sample quality (g).

2.3 Data Analysis

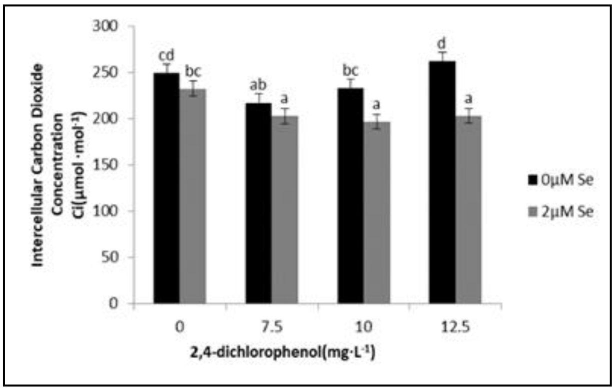
The data were processed and graphed by IBM SPSS Statistics 19.0 and Microsoft Excel 2010. The data (results) of different processing were analyzed by one-way AVOVO and Duncan multiple comparisons.

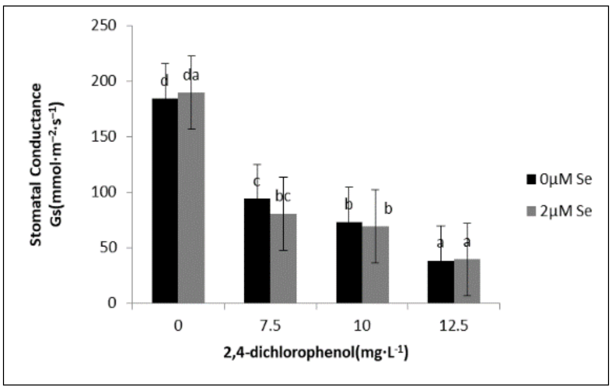
3. Results

3.1 Effects of 2, 4-dichlorophenol on photosynthetic parameters of *Salix matsudana*

Photosynthetic parameters are the most direct and obvious indicators reflecting plant photosynthesis and photosynthetic mechanism. The data showed that with the increase of 2, 4-dichlorophenol concentration, the net photosynthetic rate (Pn) of leaves of all treatments showed a significant downward trend (Fig 1). The control group had the highest net photosynthetic rate and the 12.5 mg· L^{-1} treatment had the lowest net photosynthetic rate. At the same time, compared with the treatment with and without selenium at the same concentration, it was found that the net photosynthetic rate (Pn) of each treatment with selenium was larger than that of the non-selenium treatment groups at the same concentration

The concentration of intercellular carbon dioxide (Ci) decreased in the range of 2, 4-dichlorophenol 7.5 mg·L^{-1} to 10 mg·L^{-1}, and then increased gradually with the increase of 2, 4-dichlorophenol concentration. When the concentration of 2, 4-dichlorophenol was 7.5 mg·L^{-1}, the concentration of intercellular carbon dioxide (Ci) decreased to the lowest level, and then increased slowly. At the same time, the analysis of selenium treatment group showed that the intercellular carbon dioxide concentration of Se treatment group was lower than that of selenium -free treatment group at the same concentration. In addition, it was found that the selenium treatment group and selenium-free treatment group had the same decreasing trend within the concentration range of 2, 4-dichlorophenol 0 to 7.5 mg·L^{-1}. However, the intercellular carbon dioxide concentration (Ci) of the treated plants decreased first and then increased in the range of 7.5 to 12.5 mg ·L^{-1}, but it was not significant. With the increase of 2, 4-dichlorophenol concentration, the transpiration rate (E) of plants decreased. At the same time, the transpiration rate of selenium-treated group was slightly higher than the control, but under the stress of 2, 4-dichlorophenol inforced treatment group, transpiration rate (E) of selenium treatment group was lower than that of non-selenium treatment group. Stomatal conductance (Gs) decreased with the increase of 2, 4-dichlorophenol concentration. In the group without 2, 4-dichlorophenol stress, the Gs of selenium-treated group was slightly higher than that of non-selenium-treated group, but in the group with 2, 4-dichlorophenol concentration, the transpiration rate of selenium-treated group was slightly higher than that of non-selenium treatment group. At the same time, the stomatal conductance (Gs) of selenium-treated group decreased sharply from 0 to 7.5 mg·L^{-1}, and then there was a decreasing trend in the stomatal conductance (Gs), but the decreasing speed was not significant. The reduction rates of transpiration rate (E) and stomatal conductance (Gs) in the selenium-treated group were much higher than those in the selenium-free group at the concentrations of 2, 4-dichlorophenol (0 mg·.L^{-1} to 7.5 mg·.L-1). Therefore, it is speculated that selenium treatment may enhance the stress resistance, but this enhancement may be risky in low concentration pollution stress. With the increase of pollutant concentration, plants may be more vulnerable to damage symptoms in the short term due to some performance of light and physiological enhancement.





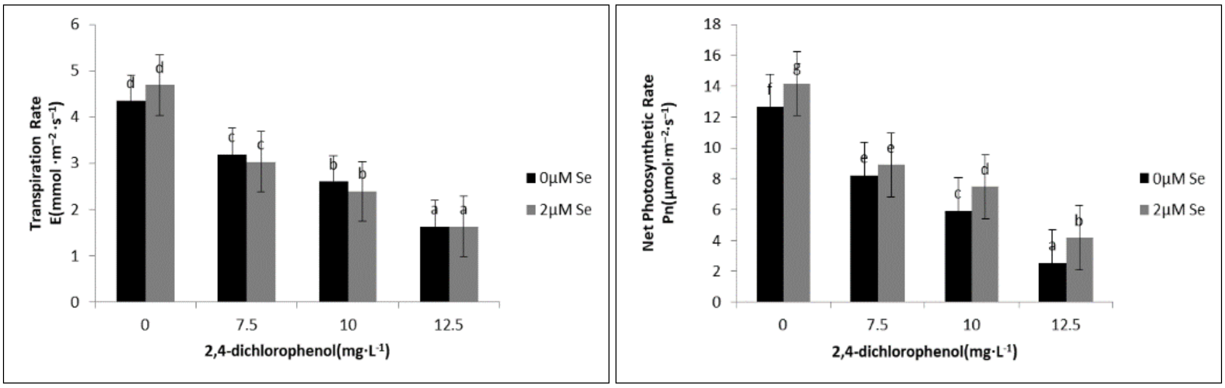


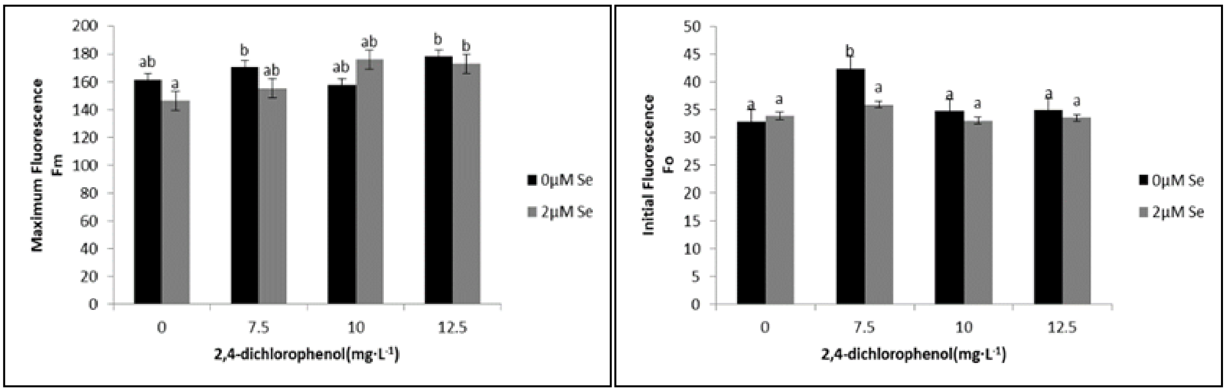
Fig 1: Changes of photosynthetic parameters of *Salix matsudana* under different concentrations of 2, 4-dichlorophenol stress and selenium addition (mean±standard error)

Different lowercase letters showed significant difference from each other ($p<0.05$). Ci: intercellular CO_2 concentration; Gs: stomatal conductance; E: transpiration rate; Pn: net photosynthetic rate.

3.2 Effects of 2, 4-dichlorophenol on fluorescence parameter of *Salix matsudana*

Fluorescence parameters can effectively reflect the photosynthetic mechanism and physiological status of plants. Analysis of fluorescence parameters can show the photosynthetic physiological level of plants in this environment. Stress treatment often changes the photosynthesis and mechanism of plants in varying degrees. Therefore, by analyzing the changes of fluorescence parameters, we can see whether 2, 4-dichlorophenol can exert a stress and inhibit the growth of *Salix matsudana* and how large the effect is. The maximum photochemical efficiency Fv/Fm curve showed a downward trend in the concentration range of 2, 4-dichlorophenol from 0 to 7.5 $mg\cdot L^{-1}$, followed by an upward trend in the concentration range of 7.5 to 12.5 $mg\cdot L^{-1}$ (Fig. 2). Compared with the same concentration of 2, 4-dichlorophenol, the maximum photochemistry efficiency of selenium-treated and non-selenium-treated plants in the control group was slightly lower than that in the non-Selenium group. With the increase of 2, 4-dichlorophenol concentration, it was found that the maximum photochemistry efficiency of selenium-treated plants was slightly higher than that of non-selenium-treated plants. The photochemical quenching coefficient qP of plants in 2, 4-dichlorophenol concentration 0 to 7.5 $mg\cdot L^{-1}$ treatment without selenium had a short rise process. Then, with the increase of 2, 4-dichlorophenol concentration, the photochemical quenching coefficient qP of plants began to decline persistently. While in selenium treatment, the photochemical quenching coefficient qP of plants descended in low 2, 4-dichlorophenol concentration and ascended in high 2, 4-dichlorophenol concentration. However, the NPQ of plants in the treatment of 2, 4-dichlorophenol concentration 0 to 7.5 $mg\cdot L^{-1}$ showed a short decline process, and then gradually increased with the increase of 2, 4-dichlorophenol concentration, and the NPQ of plants in the treatment of adding selenium and without selenium was lower than that in the treatment of adding selenium and without selenium group. However, the maximum fluorescence coefficient Fm, the actual photochemical efficiency and the initial fluorescence efficiency Fo did not change significantly, which indicated that with the increase of the concentration of 2, 4-dichlorophenol

the activity of photosystem II-related enzymes in plants would decrease, but the inhibition was not significant at low concentration. It is presumed that a concentration of 2, 4-dichlorophenol above 12.5 $mg\cdot L^{-1}$ may lead to plant death due to a sharp decline in plant enzymatic activity.



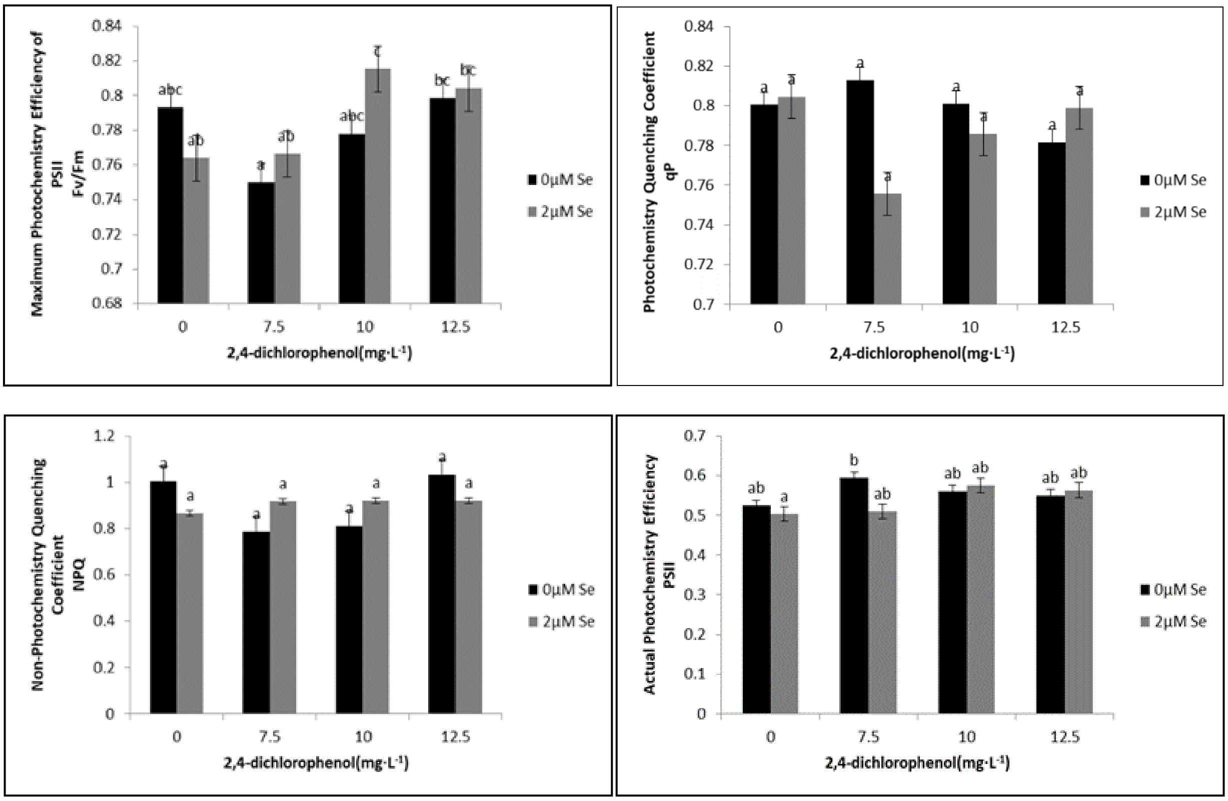
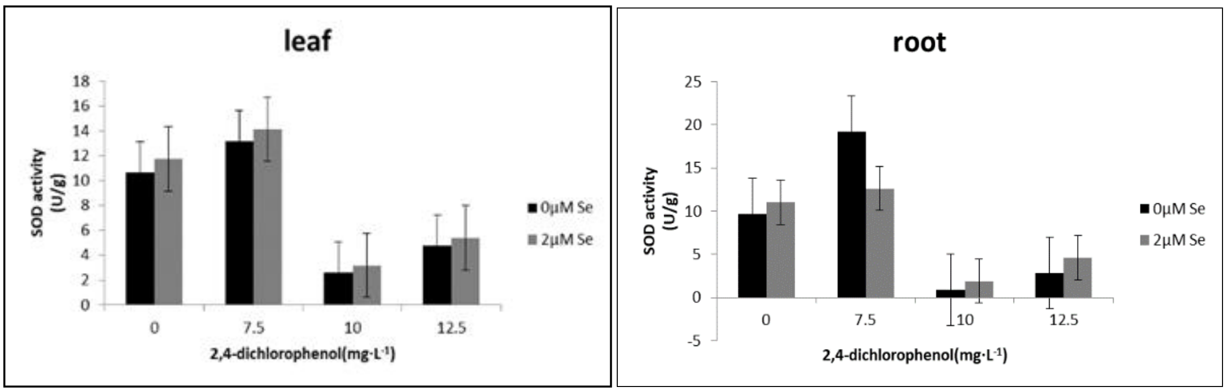


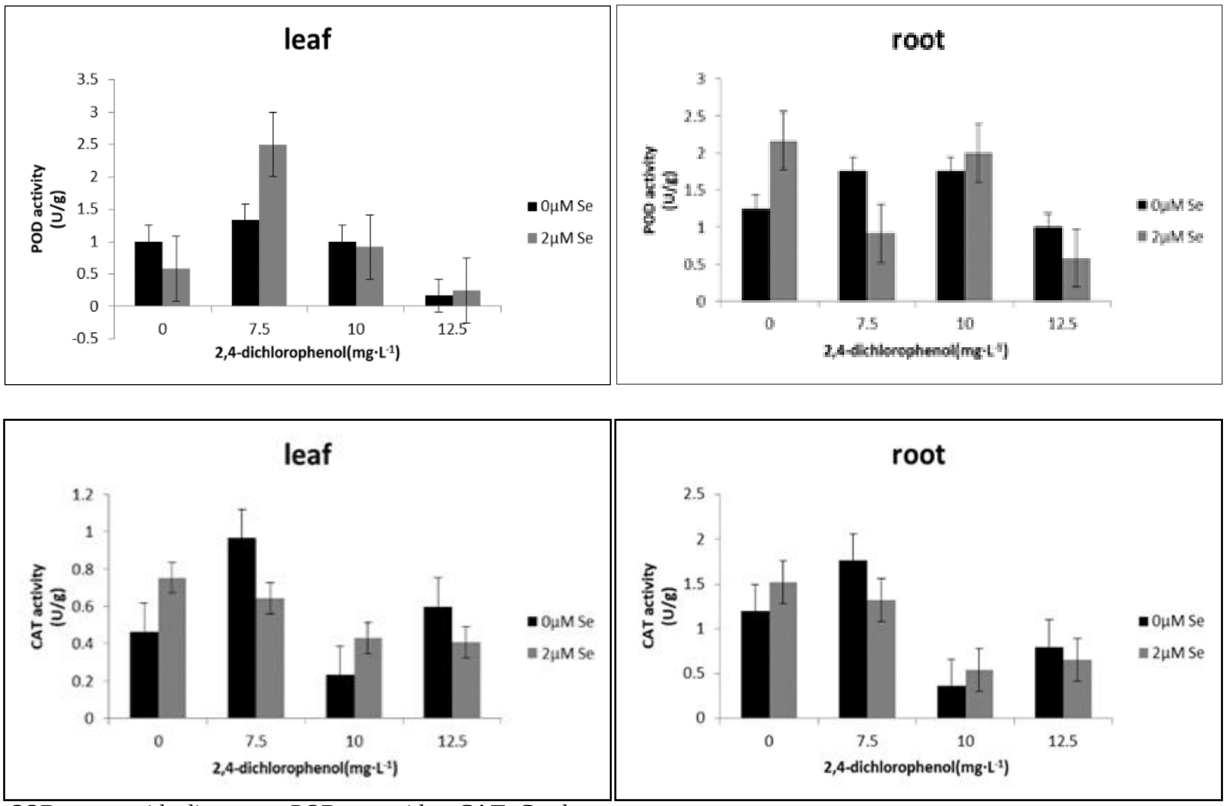
Fig 2: Changes of fluorescence parameters of *Salix matsudana* under different concentrations of 2, 4-dichlorophenol stress and selenium addition (mean±standard error)

Different lowercase letters showed significant difference ($p<0.05$). Fm: maximum fluorescence; Fo: initial fluorescence; Fv/Fm: maximum photochemistry efficiency of PSII; qP: photochemistry quenching coefficient; NPQ: non-photochemistry quenching coefficient; PSII: actual photochemistry efficiency

3.3 Effects of 2, 4-dichlorophenol on enzyme activity of *Salix matsudana*

The activity of SOD, POD and CAT increased with the increase of 2, 4-dichlorophenol concentration in the range of 0 mg·L^{-1} to 10 mg·L^{-1}. When the concentration of 2, 4-dichlorophenol was higher than 10 mg·L^{-1}, the activity of SOD, POD and CAT began to decrease gradually. This indicated that low concentration of 2, 4-dichlorophenol could obviously promote the activity of SOD, POD and CAT in s *Salix matsudana*, while the high concentration of 2, 4-dichlorophenol would inhibit the activity SOD, POD and CAT in *Salix matsudana*.



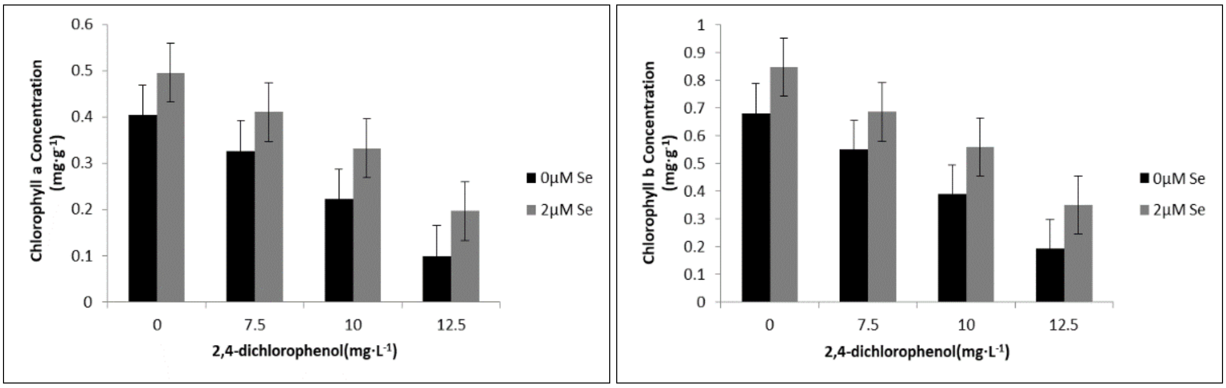


SOD: superoxide dismutase; POD: peroxidas; CAT: Catalase.

Fig 3: SOD, POD, CAT Activity of *Salix matsudana* under different concentrations of 2, 4-dichlorophenol Stress and Selenium addition.

3.4 Effects of 2, 4-dichlorophenol on chlorophyll content of *Salix matsudana*

Fig. 4 showed that with the increase of 2, 4-dichlorophenol concentration, the chlorophyll a concentration, chlorophyll b concentration, total chlorophyll concentration and total chlorophyll content of plants showed a significant downward trend. This indicated that 2, 4-dichlorophenol could significantly reduce chlorophyll content and consequently inhibit photosynthesis of plants, and the toxicity was positively correlated with 2, 4-dichlorophenol concentration. It was found that the chlorophyll a concentration, chlorophyll b concentration, total chlorophyll concentration and total chlorophyll content of plants treated with selenium at the same concentration of 2, 4-dichlorophenol were higher than those treated without selenium. This indicated that the treatment of 2 μmol·L^{-1} with selenium could increase chlorophyll content, photosynthesis and stress resistance of plants.



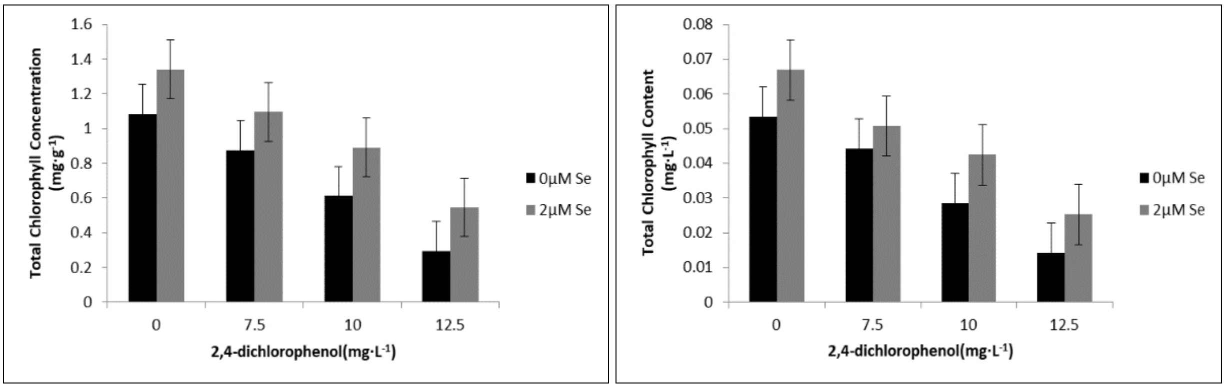


Fig 4: 2, 4-dichlorophenol stress and selenium treatment on chlorophyll content of *Salix matsudana*

4. Discussion

For plants, photosynthesis is the basis of their own survival and growth, but drought, low temperature, weak light, pollution and other stress will reduce the photosynthesis of plants [21]. Under environmental stress, the limiting factors of photosynthesis can be divided into stomatal factors and non-stomatal factors, the former refers to the closure of stomata caused by various stresses and the obstruction of CO_2 supply; the latter refers to the increase of gas phase space and diffusion resistance of CO_2 in mesophyll cells, the decrease of PSII and photophosphorylation activity, the decrease of RuBP carboxylase and FBPase activity, the obstruction of RuBP regeneration, the change of light and dark respiration, etc [22]. However, there is no unified conclusion about the effect of selenium on plants, and whether selenium is a necessary element for plants has not yet been determined. However, according to previous experiments, it was found that increasing selenium supply in selenium-deficient soils would increase plant output. At present, it is generally believed that low concentration of selenium can promote plant growth and increase plant resistance to stress, while high concentration of selenium can produce toxic effects on plants, reduce plant photosynthesis and even lead to the inactivation or death of various enzymes in plants. In addition to different concentrations of selenium will have different effects on the same plant, the same concentration of selenium will also have different effects on different plants, different plants have different resistance to and demand for selenium [23].

This experiment found that (Fig. 1) with the increase of 2, 4-dichlorophenol concentration, the net photosynthetic rate of plants showed a significant downward trend, which implied that 2, 4-dichlorophenol would have a toxic effect on plants, making their photosynthesis decreased, and with the increase of 2, 4-dichlorophenol concentration, its toxicity to plants became stronger. In addition to the net photosynthetic rate, stomatal conductance and transpiration rate also decreased gradually, indicating that 2, 4-dichlorophenol may destroy the photosynthetic structure. While the concentration of intercellular carbon dioxide decreased first and then increased gradually, indicating that the structure of photosynthetic system and the corresponding enzymes activities of potential plants have been reduced, and the photosynthesis of plants has weakened, so that they can not make full use of carbon dioxide. At the same time, through horizontal comparison, it was found that the net photosynthetic rate of Salix xerophylla was enhanced by 2 μmol $\cdot L^{-1}$ selenium concentration, so it can be considered that the concentration of selenium could enhance the photosynthetic capacity and stress resistance of Salix.

Chlorophyll fluorescence parameters play a unique role in determining the absorption, transmission, dissipation and distribution of light in plant photosynthesis [24]. Compared with the gas exchange parameters reflecting the apparent nature of photosynthesis, chlorophyll fluorescence parameters have more intrinsic characteristics reflecting photosynthesis. The increase of plant initial fluorescence (F0) can explain the decline of plant photosynthetic potential and activity to a certain extent. In Fig. 2, it can be found that the initial fluorescence of plants increased to a certain extent. At the same concentration of 2, 4-dichlorophenol, the increase of initial fluorescence in the selenium-added group is less than that in the non-selenium-added group, which proves that the selenium concentration of 2μmol$\cdot L^{-1}$ and the selenium-added treatment are indeed strengthened plant to some extent. The stress resistance of the plant was improved. From the overall level, although the fluorescence parameters measured fluctuate to a certain extent, there is no significant change between them, and there is no significant change. Therefore, this may mean that the 2, 4-dichlorophenol stress concentration selected in the experiment has not yet caused great damage to the photosynthetic system of *Salix matsudana*.

Chlorophyll is an important indicator of plant growth. According to Fig. 4, with the increase of 2, 4-dichlorophenol concentration, the chlorophyll content of plants decreases accordingly, which indicates that 2, 4-dichlorophenol does have some negative effects on plants, it inhibits the chlorophyll content of plants and then reduces the photosynthesis of plants.

The activities of SOD, POD and CAT in plants can reflect the degree of stress and the potential damage level of its own mechanism to a certain extent. According to Fig. 3, it was found that the activity of corresponding enzymes in plants increased with the increase of 2, 4-dichlorophenol concentration at low concentration. It can be inferred that a large number of free radicals were produced in plants due to environmental stress, and the enzymatic reaction mechanism of plants themselves produced a large number of enzymes to eliminate harmful free radicals. However, when the concentration of 2, 4-dichlorophenol was higher than 10 mg$\cdot L^{-1}$, the activity of plant enzymes began to decrease, which indicated that the plant enzymatic system may have been destroyed. It can be inferred that with the increase of the concentration of 2, 4-dichlorophenol, the activity of corresponding enzymes in plants will continue to increase to eliminate free radicals, and then the activity of enzymes will decrease until it disappears due to the high concentration of 2, 4-dichlorophenol.

5. Conclusion

In conclusion, 2, 4-dichlorophenol can cause toxicity to *Salix matsudana*, and the toxicity will be strengthened with the increase of 2, 4-dichlorophenol concentration. At the concentration of 12.5 mg·L^{-1}, the photosynthesis and growth of plants were inhibited, but they could still grow at this concentration. The inhibition of photosynthesis by 2, 4-dichlorophenol is due to non-stomatal factors. When the concentration of 2, 4-dichlorophenol is higher than a certain value, the photosynthetic system of *Salix matsudana* will be damaged obviously. Therefore, *Salix matsudana* has a good resistance to 2, 4-dichlorophenol. Combining with the research done by Shi *et al*. [25], the purification effect of *Salix matsudana* on 2, 4-dichlorophenol is also at a relatively high level. Therefore, *Salix matsudana* can be used to remediate 2, 4-dichlorophenol-contaminated water or soil in real life. At the same time, measures such as selenium addition can be used to improve plant growth and stress resistance, while counteracting some toxic effects such as photosynthesis decline after stress.

6. 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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