

The response of Antioxidant enzymes and Photosynthesis dynamics of Sunflower Exposed to Aniline Wastewater

ABSTRACT

Aims: In order to investigate the tolerance of sunflower to aniline, we measured the photosynthetic parameters, chlorophyll fluorescence parameters and antioxidant enzyme activities of sunflower in aniline wastewater.

Study design: The experiment was conducted with oil sunflower No. 4 (*Helianthus annuus* Linn.) as the experimental material. After 5 weeks of culture in Hoagland medium of 50% concentrations, seedlings with uniform growth were selected for aniline stress treatment.

The concentrations of aniline stress treatment were 60, 80, 100, 120, 140, 160 and 180 mg /L, respectively. The control was without aniline, and 8 replicates per treatment. Leaf photosynthetic parameters were measured under natural environmental conditions 5 and 10 days after treatment.

Place and Duration of Study: Place of study was Shandong Agricultural University, Department of Taian, Shandong, China and experiment lasted for 10 weeks.

Results: Five days after stress treatment, the net photosynthesis rate (P_n), stomatal conductance (G_s), and transpiration rate (T_r) of sunflower showed a upward trend at the lower concentration of aniline (≤ 100 mg/L) and turned to a downward trend at higher concentration of aniline (≥ 120 mg/L). However, ten days after stress treatment, P_n , G_s and T_r of sunflower decreased significantly ($P=0.05$) compared to the control in all concentrations. The photochemical quenching coefficient and effective quantum yield of PSII photochemistry decreased. At the end of the ten day experiment, with the increase of aniline concentration, the photosynthetic and chlorophyll fluorescence parameters of sunflower showed an downward trend except intercellular CO_2 concentration. The data indicated that nonstomatal limitation was responsible for the reduction of P_n . When the aniline concentration reached 180 mg/L, the activity of SOD and POD reached the highest point, which was 3.49 times and 1.78 times higher than that of the control.

Conclusion: In short, the low concentration of aniline (<120 mg / L) could promote the normal growth of sunflower in a short time. However, when the stress time reaches a certain level, aniline will have a significant toxic effect on sunflower, seriously affecting the normal growth of sunflower.

Keywords: aniline stress; photosynthesis; sunflower; SOD; POD

1. INTRODUCTION

Aniline is the most representative substance of aromatic amines, it is one of the main pollutants in the printing and dyeing industry, pharmaceutical industry, agriculture and other industries in China. With the development of these industries, the emission of aniline in environment increases every year. Aniline has strong toxicity and inhibits the growth of aquatic animals and plants. Aniline enters the body mainly through the skin, the respiratory tract, and the digestive tract, it can cause hemolytic anemia and toxic hepatitis. For these reason, China has included aniline in the blacklist of China's environmental priority pollutants, and the United States EPA also ranked aniline as one of the 129 priority pollutants[1,2].

Compared with the commonly used physical methods, chemical methods and microbial remediation methods, phytoremediation can not only remove pollutants but also promote the recycling and reuse of nutrients in sewage. And it can green environment, improve regional climate, promote the virtuous cycle of the ecological environment[3]. Liu[4] uses the wheatgrass (*Agropyron cristatum* (L.) Gaertn.) to carry on the plant restoration experiment to the aniline contaminated soil, discovered that the ice

grass root system can absorb the aniline, passes through the stem to the leaf and the panicle transport, Wang and Wen[5] found that the efficiency of phytoremediation of aniline in water was 50.7%~97.3% and water spinach (*Ipomoea aquatica* Forsk) had the best repair effect.

Sunflower (*Helianthus annuus*), Compositae sunflower genus, is a kind of salt resistance and drought resistance barren, adaptability, oil crops[6-8]. Zhao[9] compared the remediation effects of soil uranium pollution on 5 plants, found the sunflower (*Phaseolus vulgaris* L) and sauteed green beans as phytoremediation of soil polluted by uranium sequestration. Some sites in the United States used sunflowers to repair radionuclides contaminated groundwater[10]. Xie[11] founds that sunflower had different tolerance and decoloration rates to different kinds of azo dye wastewater. Therefore, sunflower has good environmental adaptability, foundation and potential in the purification of Aniline Wastewater. The photosynthetic physiological responses of sunflower to aniline wastewater have not been reported yet. In this study, soil sunflower No. 4 was used to analyze the effects of different concentrations of aniline stress on photosynthesis, chlorophyll fluorescence parameters and antioxidant enzyme activities of sunflower. The aim of this study was to reveal the tolerance and adaptation mechanism of sunflower to aniline stress and to provide scientific basis for phytoremediation of aniline in water.

2. MATERIAL AND METHODS

2.1 Experimental design

The experiment was conducted with oil sunflower No. 4 (*Helianthus annuus* Linn.) as the experimental material. The seeds of plump and the same size were selected. After 24 hours soaking, the seeds were buried in sterilized quartz sand, watered with deionized water, and germinated under natural conditions. Healthy seedlings were selected and placed in 250 mL volumetric flasks. After 5 weeks of culture in Hoagland medium of 50% concentrations, seedlings with uniform growth were selected for aniline stress treatment.

The concentrations of aniline stress treatment were 60, 80, 100, 120, 140, 160 and 180 mg /L, respectively. The control was without aniline, and 8 replicates per treatment. Leaf photosynthetic parameters were measured under natural environmental conditions 5 and 10 days after treatment.

2.2 Items and methods of measurement

2.2.1 Determination of photosynthetic physiological parameters

Under the shiny weather condition, three mature leaves were selected for the determination of photosynthetic parameters from the middle part of each seedling. The net photosynthetic rate(P_n), transpiration rate(T_r), stomatal conductance(G_s), intercellular CO_2 concentration(C_i) were measured by the CIRAS-2 photosynthesis system produced by the British PPS company. Water use efficiency(WUE) and stomatal limitation(L_s) were calculated by formula:

$$WUE = P_n / T_r [12], L_s = 1 - C_i / C_a [13]$$

Where C_a is atmospheric CO_2 concentration

2.2.2 Chlorophyll fluorescence parameter determination

After 30 min activation in natural light, minimal fluorescence yield of the light-adapted state(F_o'), maximal fluorescence yield of the light-adapted state(F_m') and steady-state fluorescence yield(F_s) of sunflower leaves under different aniline concentration gradients were measured using a pulsed modulated fluorescence system (FMS2.02 type, Hansatech, UK). The minimal fluorescence(F_o) and maximal fluorescence(F_m) were measured after 30 min dark adptation. Optimal/maximal photochemical efficiency of PS in the dark was thus calculated use:

$$F_v / F_m = (F_m - F_o) / F_m [14]$$

actual photochemical efficiency of PS in the light was calculated use

$$\Phi_{PSII} = (F_m' - F_s) / F_m' [14]$$

photochemical quenching was calculated use

$$qP = (F_m' - F_s) / (F_m' - F_o') [15]$$

nonphotochemical quenching was calculated use

$$NPQ = (F_m - F_m') / F_m' [15]$$

2.2.3 Enzyme activity assay

At the end of the experiment, sunflower leaves were taken, washed with distilled water, dried with absorbent paper, and the enzyme activity was measured. Determination of SOD and POD activity was performed by the method of Liu Ping[16].

2.3 Data analysis

SPSS 17.0 software was used for data analysis, and Excel 2010 software was used for data analysis and mapping. The difference of between control was analyzed by one-way analysis of variance(Anova) and Duncan's multiple-range test was used for multiple comparisons.

3. RESULTS

3.1 Effects of aniline stress on photosynthetic parameters of sunflower

The change of photosynthetic parameters after 5days 10 days exposure to aniline are presented in Fig.1. In fifth day, the net photosynthetic rate (P_n) of sunflower leaves showed increase trend at lower aniline concentration (≤ 100 mg/L) and decline trend at higher aniline concentration (≥ 120 g/L). The P_n with aniline concentration of 100 mg/L was highest ($28.77 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), 1.28 times as high as that of the control group ($P=0.05$). When the aniline concentration reached 140 mg/L, the P_n of sunflower decreased significantly ($P=0.05$). When the aniline concentration reached 180 mg/L, P_n was only 66.88% of the control group. The results showed that low concentration of aniline promoted the photosynthesis of sunflower, and high concentration of aniline inhibited photosynthesis, the inhibitory effect increased with the increase of concentration. With the increase of aniline concentration, the stomatal conductance (G_s), transpiration rate (T_r) and stomatal limitation value (L_s) were the same as those of P_n , all showed the trend of increasing at first and then decreasing. The change trend of C_i is opposite to that of P_n . When the concentration of aniline is greater than 100 mg/L, with the increase of aniline concentration, L_s decreases significantly, while C_i increases significantly($P=0.05$). The main reason for the decline of P_n was the change from stomatal factor to non stomatal factor. When the experiment was carried out for ten days, with the increase of aniline concentration, the P_n , G_s , L_s , T_r and WUE of sunflower showed a monotone trend, while C_i showed an upward trend. When the concentration of aniline was 60 mg/L, the P_n , G_s , L_s , T_r and WUE in the experimental group were significantly lower than those in the control group ($P=0.05$), while the trend of C_i was contrary to that of P_n . Compared with the fifth day test, the P_n , G_s , L_s , T_r and WUE in the experimental group were significantly decreased. When aniline concentration is 100 mg/L, the reduction of P_n is the maximum, and it is 12.28% of the value of fifth day.

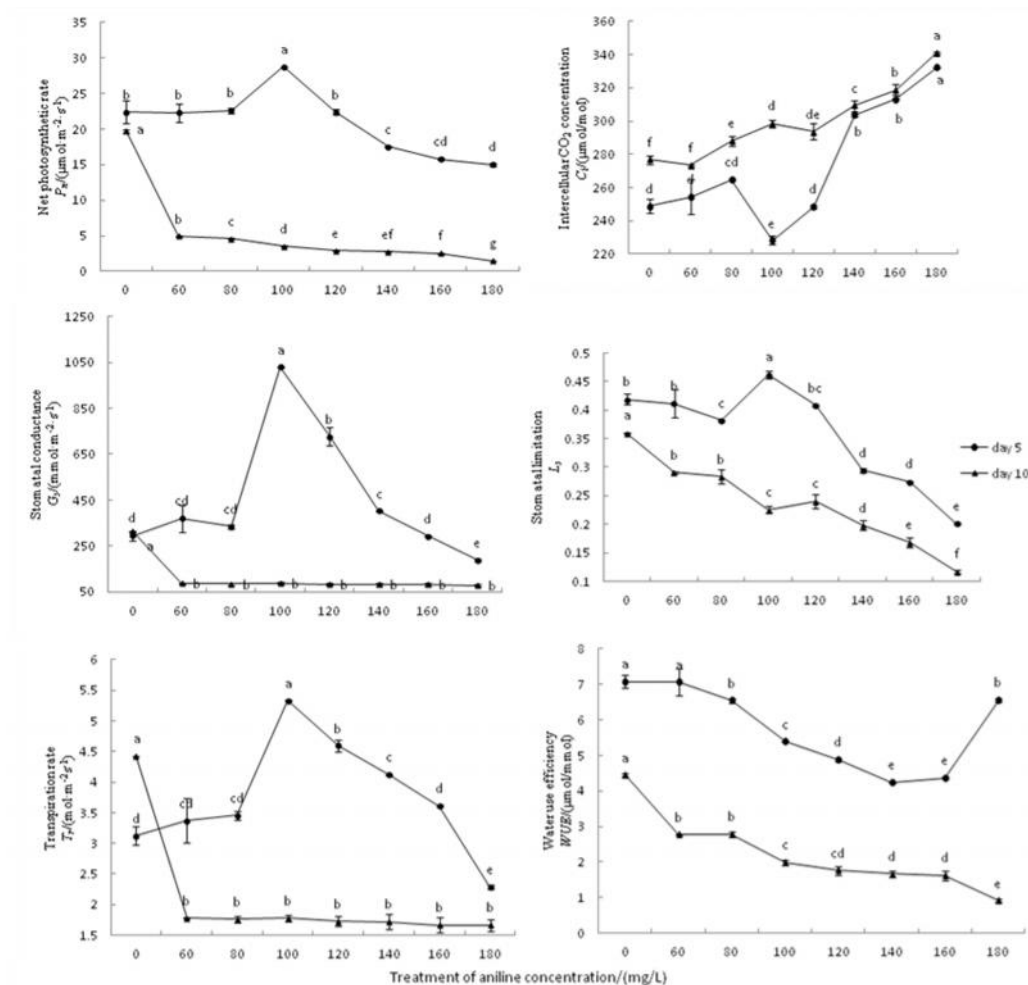


Fig. 1. Photosynthetic parameters of sunflowers under aniline stress

Test drugs: significant from normal control, different lowercase letters $P < 0.05$

Mean $\pm$ S.E.M = Mean values $\pm$ Standard error of means

3.2 Effect of aniline stress on fluorescence parameters of sunflower

In the fifth day, with the increase of the concentration of aniline, Φ_{PSII} and qP were significantly decreased and the maximum chemical efficiency (F_v/F_m) of PSII decreased significantly when the concentration of aniline was 160 mg/L. The non photochemical quenching coefficient (NPQ) was significantly higher than that of the control group only when the concentration of aniline was 60 mg/L ($P=0.05$). When the concentration of aniline was greater than 60 mg/L, there was no significant difference between the control group and the control group ($P>0.05$). In the tenth day, with the increase of the concentration of aniline, qP , PS II, F_v/F_m and NPQ were significantly lower than the control group ($P=0.05$). The decline of F_v/F_m and PS II showed a decrease of the potential activity of PS II, and the decrease in qP showed a reduction in activity of the PS II reaction center[17]. The results showed that aniline stress could obviously inhibit or decrease the photochemical activity and photochemical efficiency of PS II and the longer stress lasted the more serious the stress effect.

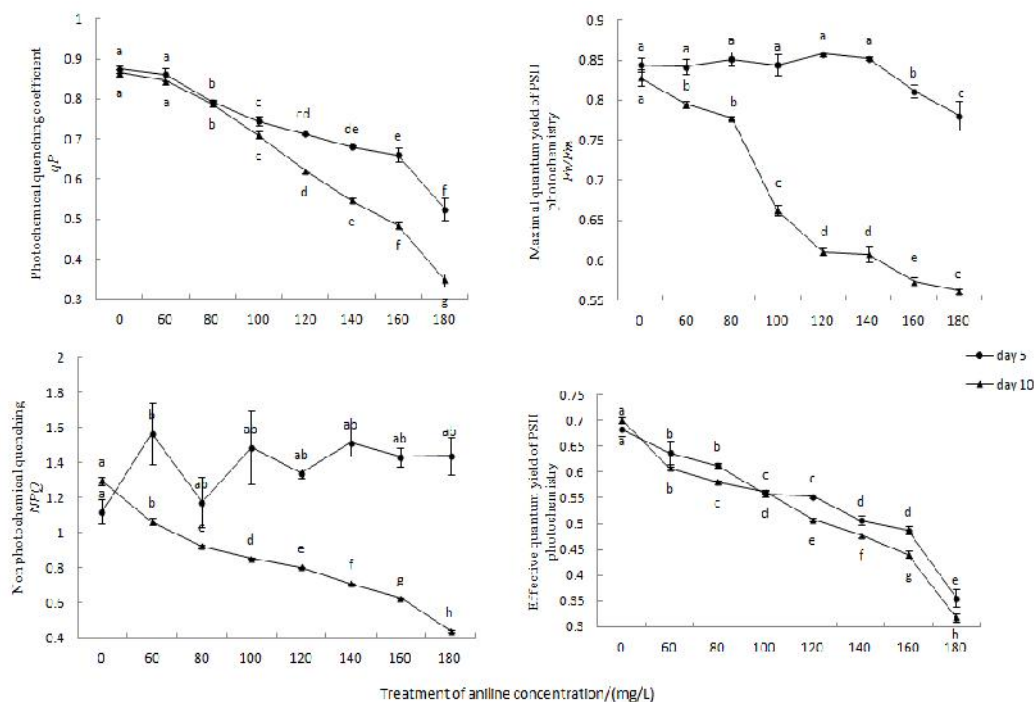


Fig. 2 Fluorescence parameters of sunflowers under aniline stress

Test drugs: significant from normal control, different lowercase letters $P < 0.05$

Mean $\pm$ S.E.M = Mean values $\pm$ Standard error of means

3.3 Effect of aniline stress on SOD and POD

The activity of protective enzymes SOD and POD is closely related to the resistance of plants. As it can be seen from Figure 3, with the increase of aniline concentration, the SOD and POD activity of sunflower were on the rise, when aniline concentration reached 180 mg/L, the activity of SOD and POD reached the highest point, respectively, 3.49 times and 1.78 times of the control.

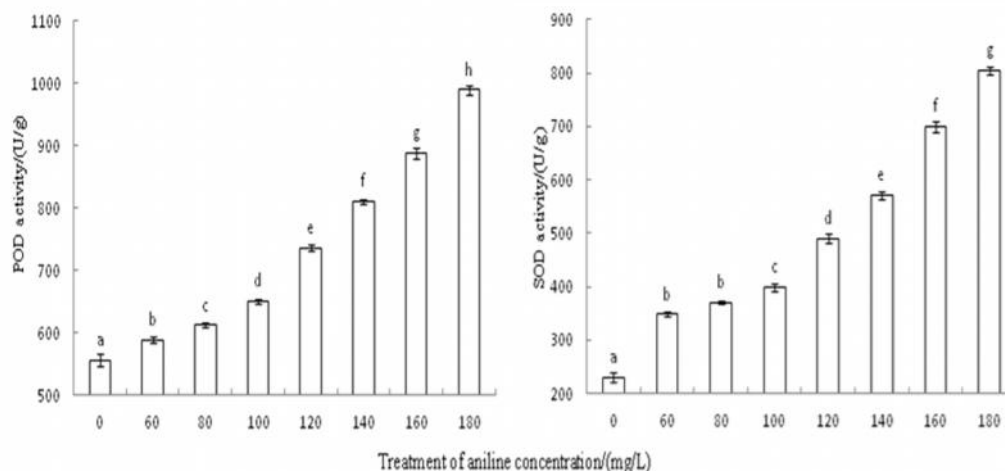


Fig. 3 Activity of SOD and POD of sunflowers under aniline stress

Test drugs: significant from normal control, different lowercase letters $P < 0.05$

Mean $\pm$ S.E.M = Mean values $\pm$ Standard error of means

4. DISCUSSION AND CONCLUSION

Photosynthesis is the basis of plant growth, while stress has more obvious effects on Photosynthetic Physiology of plants. Zhang[18] found that low temperature, weak light and salt stress can reduce net photosynthesis of pepper, and the inhibition effect of compound stress is greater than that of single stress. Generally, the factors that affect plant photosynthesis include stomatal factors and non stomatal factors, the former refers to water stress leading to stomatal conductance decline, CO₂ into the leaves blocked and photosynthetic decline, the latter refers to the photosynthetic activity of mesophyll cells decreased[19]. The present study shows (Fig. 1) when the test is carried out to fifth days, when the concentration of aniline was 100 mg/L, the net photosynthetic rate of sunflower was significantly higher than that of the control ($P=0.05$). It was suggested that low concentration of aniline could promote the physiological activities of sunflower. When the aniline concentration reached or exceeded 140 mg/L, aniline inhibited the photosynthesis of sunflower obviously, the greater the concentration, the stronger the inhibition ability. The decline in P_n was accompanied by a rise in C_i and a fall in L_s , the main limiting factor for photosynthesis at this time was non stomatal factors[20], the photosynthetic apparatus of sunflower was damaged. Li Hui [21] found that in the relatively low concentration of phenol (50 mg/L) net photosynthesis of salix seedlings decreased. Xie [11] showed that the net photosynthetic rate of sunflower in three kinds of azo dyes (Bismarck brown, Evans blue, orange) at a concentration of 100 mg/L decreased significantly. Wang's [22] study showed that the low net concentration of naphthalene (10 mg/kg) increased the net photosynthetic rate, but high concentrations were markedly inhibited. However, the same concentration of 1,2,4-trichlorobenzene could significantly inhibit the net photosynthetic rate of rice. The above studies show that the toxicity of organic matter to plants is very different, The photosynthetic physiological response of plant to organic pollution stress also showed difference. When the aniline stress time reached 10 days, the multiple photosynthetic indexes of sunflowers (P_n , Tr , G_s , L_s , WUE) were significantly lower than those in the control group ($P=0.05$), and The gap between test indexes on the fifth day is relatively large. The normal physiological activity of sunflowers can be seriously affected even if the aniline stress is prolonged, even if the concentration of aniline is not high (60 mg/L).

Chlorophyll fluorescence parameters play a unique role in determining the absorption, transmission, dissipation and distribution of light energy during photosynthesis of plants[20]. F_v/F_m is PS II maximum quantum yield of photochemical reaction, reflects the PS II reaction center of light energy conversion efficiency [23], the parameter change is very small under the condition of non-stress, not affected by species and growth condition, and the parameter significantly decreases under stress. By the fifth day of the experiment, when the concentration of aniline is less than 160 mg/L, the data in the experimental group and the control group were not different significantly (Fig. 2), this suggests that in a short time, low concentration of aniline stress had no serious effect on the sunflower PSII system. FPS II reflects the proportion that light energy absorbed by the photosynthetic apparatus for photochemical reactions in the sun[24]. QP indicates that how the excitation energy used by PSII reaction center, the higher the value, the higher the electron transfer activity of PSII[25]. This experiment shows that (Fig. 2), as the concentration of aniline increases, qP and FPS II all showed a trend of decline, it means that aniline stress increases the PS II reaction center close degree and the photosynthetic electron transport rate goes down, The light energy for PS II actual chemical reaction has been reduced[24]. With the increase of aniline concentration, NPQ showed a wave trend and increased significantly compared with the control group only when the concentration of aniline was 60 mg/L ($P=0.05$), This shows that the sunflower's light damage defense mechanism starts, the light energy absorbed by PSII of which the amount of heat dissipation which use by antenna pigment increases, thus help ease the destruction of PS II reaction center institutions caused by aniline stress[26]. In the tenth day of the antenna pigment, the F_v/F_m decreased significantly when the concentration of aniline was 60mg/L, reflects that there is a photoinhibition in the photosynthesis of sunflower leaves or the PS II system was destroyed. The NPQ decreased with the increase of aniline concentration, shows that the amount of heat dissipation which use by antenna pigment descends too and prolonged aniline stress may have an effect on Violaxanthin de-Epoxidase, caused the heat dissipation decreases which depends on the lutein cycle[24], sunflower PS II photosynthetic system suffer obvious injury by bright light. The research of Zeming Wang [22] shows that as the concentration of Naphthalene and 1, 2, 4-trichlorobenzene in soil increases, NPQ decreases rapidly, and the self-protection mechanism of rice through thermal dissipation has been seriously damaged.

Under normal circumstances, the generation and removal of intracellular free radicals in a dynamic equilibrium state, the free radical level is very low, will not hurt the cells. However, under stress, the accumulation of reactive oxygen species in plant cells will lead to cell membrane damage and cell damage and even death [27]. Adversity induced, the plant body to start their own protection mechanism, to minimize the damage. Plant SOD, POD and CAT together constitute an antioxidant protective enzyme system, which can remove free radicals such as O_2^- , $-OH$ and so on in the plant under adverse stress, and relieve or reduce the damage degree of plants. Among them, SOD is a plant through the catalytic O_2^- -conversion to O_2 and H_2O_2 , POD will transfer H_2O_2 into O_2 and H_2O , to eliminate the harm of free radicals [28]. The increase of SOD and POD activity in this plant under the condition of aniline stress is a mechanism of plant self-protection against stress. The results showed that the activity of SOD and POD in sunflower increased with the increase of aniline concentration, which indicated that aniline stress could increase the activity of reactive oxygen species and promote the increase of SOD and POD activity. The cells produced a significant toxic effect. This is similar to that of Wen Guangjun [29] and Bai Weirong [30], indicating that low concentration of aniline can promote the increase of SOD and POD activity. Sheng's study showed that the activities of SOD and POD in sunflower showed a tendency to increase first and then decrease with the increase of salt concentration, which indicated that low concentration of salt could obviously promote the activity of SOD and POD in sunflower, while the high concentration salt will inhibit the activity of sunflower SOD and POD[31]. The activity of SOD and POD in this experiment did not decrease with the increase of aniline concentration, which indicated that the aniline concentration was 180 mg/L and could not inhibit the activity of SOD and POD in sunflower [28].

In short, the low concentration of aniline (<120 mg/L) could promote the normal growth of sunflower in a short time. When the concentration of aniline gradually increased, the photosynthetic rate of sunflower decreased. However, when the stress time reaches a certain level, aniline will have a significant toxic effect on sunflower, seriously affecting the normal growth of sunflower.

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