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Research on the Law of Stress of Polychlorinated Naphthalenes (PCNs) on the Physiological Ecology of Bluegrass (*Poa annua* L.)

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ABSTRACT

This paper takes *Poa annua* L. as the research object and studies the law of physiological and ecological stress of 1-Chloronaphthalene (CN-1) and Octachloronaphthalene (CN-75) by using various physiological and biochemical indexes of *Poa annua* L. cultivated with soil under the stress of CN-1 and CN-75 of different concentrations. According to the research, the chlorophyll *a* and *b* first increase and then decrease with the increase of the concentration of CN-1, and continue to decrease with the increase of CN-75; Soluble sugar and soluble protein in plants decrease first and then increase with the increase of CN-1, and continue to decrease with the increase of CN-75; MDA in plants increases first and then decreases with the increase of the concentration of CN-1 and CN-75. The proline content in plants also increases first and then decreases with the increase of concentration of CN-1 and CN-75. Based on the research, it can be seen that the tolerance of the plant to CN-75 is not good as to CN-1.

INTRODUCTION

PCNs are kinds of persistent organic pollutant with physical and chemical properties similar to dioxin organic matter (Liu et al. 2013) and are featured by semi-volatility, higher concentration in the air, remote transmission, relative good heat resistance, insulation, and thermal insulation properties. It cannot be oxidized and decomposed under natural conditions easily and will have persistent influences on the environment. It has low solubility in water, but the lipophilicity is better than that of the general organic matter. Industrial production generates most of the total amount of PCNs, and processes such as household waste treatment, chemicals, metal smelting, and electronics production will produce many PCNs (Hu et al. 2012).

PCNs in the environment can't be degraded easily, and are certainly enriched and bio-accumulated in organisms. People have detected PCNs in the atmosphere, water, soil and organisms, and PCNs have been a global environmental pollutant. Although PCNs are similar to other polychlorostyrene bicyclic hydrogen compounds (such as polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls) in structure and properties, PCNs have not been studied so deeply as those pollutants (Shi et al. 2014). PCNs and PCBs are certainly harmful to human, animal and plant health, and PCNs are more hazardous than PCBs (Park et al. 2010).

Plant Stress

Plants can absorb volatile organic compounds (VOCs) from the soil in high concentrations. Plant roots can not only absorb PCNs in water and soil solutions but also transfer some compounds to the above-ground parts, affecting the growth of plants.

The environment harmful to plants is called adversity, also called stress. The reveal of plant adaptability or physiological mechanism under the stress of PCNs by studying the physiological response of plants to PCNs stress can help to study the effects and stress mechanism of PCNs on organisms, and enhance the stress resistance of organisms to PCNs or protect organisms from harm, creating favorable conditions for the growth of organisms (Ma et al. 2009). At present, there are few comprehensive and systematical studies on the effects of PCNs to plant physiology and biochemistry, and on physiological and biochemical indexes with outstanding responses to plant stress (Xu & Wang 2006).

Research Status at Home and Abroad

There is less data on PCNs concentration in the atmosphere throughout the country. At current, most of the literature in China only studies the distribution, pollution sources and degradation of PCNs, and there are few studies on its absorption, accumulation, movement, and transformation in environmental media.

Different species of plants respond differently to naphthalene. For example, Liu et al. (2002) found that naphthalene with different concentrations (1.2-1.6 mg.L^{-1}) significantly reduced the respiration intensity and chlorophyll content of five plants, showing a negative relation with naphthalene content; In terms of the peroxidase (POX) activity, water hyacinth and groundnut were positively correlated with the concentration of naphthalene, while duckweed, purple duckweed, and fine leaf *Polygonum chinensis* showed a trend of first increasing and then decreasing with the concentration of naphthalene increasing. Du et al. (2006) studied the effects of 1,2,4-trichlorobenzene on rice seed germination and seedling growth and found that the plant height and root length of rice seedlings were inhibited, and showed a certain concentration-effect and time-effect relationship. Yufang et al. (2002) found that 1,2,4-trichlorobenzene can significantly inhibit root elongation of three higher plants of wheat, cabbage and tomato. In general, bioconcentration is enhanced with the increase of the number of chlorine atoms.

MATERIALS AND METHODS

Selection of *Poa annua* L.

Belonging to Gramineae, *Poa annua* L. is mainly distributed in temperate and cold regions and is rare in the tropics. China has about 100 species, mainly distributed in northern China, and they are the important components of grassland and meadow vegetation. With features such as good palatability, strong regeneration ability, rich nutrition, long green period, and good trampling resistance, it is a forage resource with great development and utilization prospects in northern China; At the same time, as the excellent cool-season lawn plant, it is widely used in various lawn establishment and is a valuable plant germplasm resource (Xie 2001).

The *Poa annua* L. is taken as the research object to treat leaves with CN-1 and CN-75 at different concentrations and to study the content changes of photosynthetic pigments, soluble sugars, soluble proteins, MDA, and proline of plants under the stress of PCNs.

In the experiment, the spectrophotometer is used to measure the absorbance at different wavelengths, and to calculate the content indirectly, and the comparison of the content of the above matters in a certain amount of organisms under normal conditions and adversity stress can help to understand changes in physiological and biochemical indexes of *Poa annua* L.

Planting of *Poa annua* L.

Poa annua L. is cultivated in brown soil. According to the plastic flowerpot's specification, the length of the basin is 20 cm, and the depth of the basin is 15 cm. Put soils of the same amount in flowerpots, and split water over them after soil compaction. Select full *Poa annua* L. seeds of the same size, and sow 250 seeds per pot. Cover the surface with fine sand, and place them on an indoor windowsill. Water them regularly after seed germination. Perform a stress simulation experiment when the *Poa annua* L. seedlings' length grows to more than 10 cm.

Select the leaves with similar size and the same growth status, then spray with a spray pot containing different concentrations of PCNs 1, 3 and 5 days before the experiments to make bluegrass grow under the stress of PCNs. The control of CN-1 is ethanol mixed with water, while the blank control of CN-75 is hypochloronaphthalene mixed with water. The concentration of the experimental group solution is CN-1 and CN-75 (10 mg.L^{-1}, 20 mg.L^{-1}, 50 mg.L^{-1}, 100 mg.L^{-1}, 200 mg.L^{-1}), CN-75 (10 mg.L^{-1}, 20 mg.L^{-1}, 50 mg.L^{-1}, 100 mg.L^{-1}, 200 mg.L^{-1}). Three parallel repeated samples are set for each treatment group and control group for the planting. Use quantitative leaves cut into pieces for pretreatment measurement and analysis.

Measurement Methods of Each Index

The photosynthetic pigments are measured by the spectrophotometric method, and the content of photosynthetic pigments can be calculated based on the absorption of visible light at a specific wavelength. This method can measure out the contents of chlorophyll a, chlorophyll b, and carotenoids without separation; The soluble sugars are measured by the anthrone mensuration, and under the action of concentrated sulfuric acid, sugars can be dehydrated to form furfural or methyl furfural which then can be reacted with anthrone to form blue-green furfural derivates. Within a certain range, the intensity of the color is directly proportional to the sugar content, which can be used for the quantitative determination of sugars. The soluble proteins are measured by the coomassie brilliant blue G-250 method which uses the principle of protein-dye combination to quantitatively determine the concentration of microproteins. The MDA is measured with the stable red product after heating the proline and ninhydrin reagent under acidic conditions, and its content is positively correlated with the color depth. There is a maximum absorption peak at 515 nm, which can be measured with a spectrophotometer.

Analysis Method

Each treatment is repeated 3 times. SPSS19.0 statistical software is used for statistical analysis of experimental data, and the figures were finished with Origin software 8.0. The statistical software is used to conduct "one-way ANOVA" of the mean of each data, and complete the difference analysis among numeric values; $p < 0.05$ indicates a significant difference; $p < 0.01$ indicates an extreme difference. Different lowercase letters in the figure indicate the significant difference between the values, and the same letters indicate no significant difference between them.

RESULTS

Photosynthetic Pigments Content

Photosynthetic pigments include chlorophyll a, chlorophyll b, carotenoids and lutein. The change of photosynthetic pigment content in plants with the change of PCNs concentration is shown in Figs. 1-4. Different lowercase letters indicate the significant difference among different concentrations, and the same letters indicate no significant difference between them.

As shown in the figures, the content of chlorophyll *a*, chlorophyll *b*, and carotenoids in *Poa annua* L. increase correspondingly with the increase of the concentration of CN-1 while the concentration of CN-1 is lower than 50 mg.L^{-1}. When the concentration of CN-1 is 50 mg.L^{-1}, the content of chlorophyll a and b in *Poa annua* L. reach the maximum, increasing by 123.7% and 129.8% respectively compared with the control. When reaching the peak, the content of chlorophyll in plants decreases with the increase of the concentration of CN-1.

The content of chlorophyll a and b decreases with the increase of the concentration of CN-75. With the increase of the concentration of CN-75, the content of carotenoids decreases first and then increases, reaching the minimum when the concentration of CN-75 is 20 mg.L^{-1}.

Soluble Sugars Content

The content of soluble reducing sugars in *Poa annua* L. under the stress of different concentrations is studied, and changes of soluble reducing sugars in plants with changes in PCNs concentration are shown in Fig. 5. There is no significant difference (P> 0.05) between the two groups with CN-1 of 0 mg.L^{-1} and CN-1 of 200 mg.L^{-1}, CN-1 of 20 mg.L^{-1} and

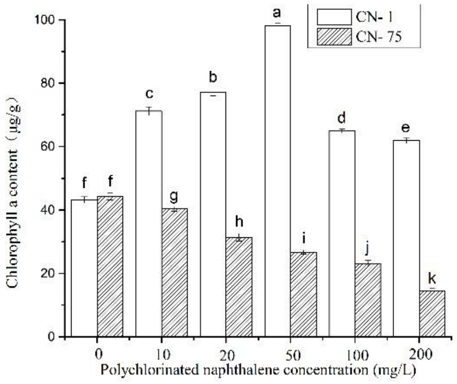

Fig. 1: Chlorophyll a content with changes in PCNs concentration.

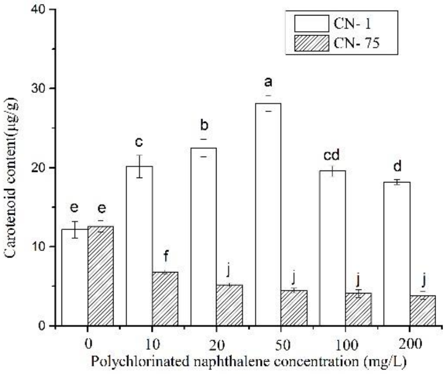

Fig. 3: Carotenoids content with changes in PCNs concentration.

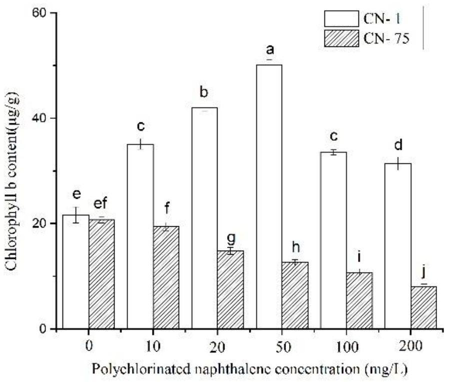

Fig. 2: Chlorophyll b content with changes.

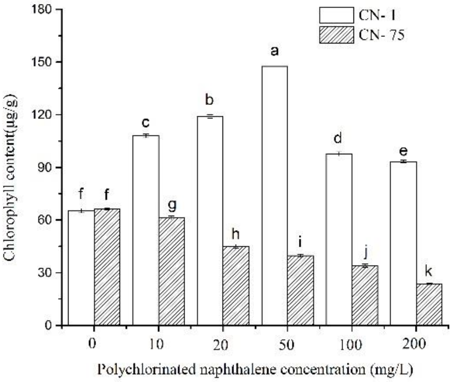

Fig. 4: Total chlorophyll content with changes.

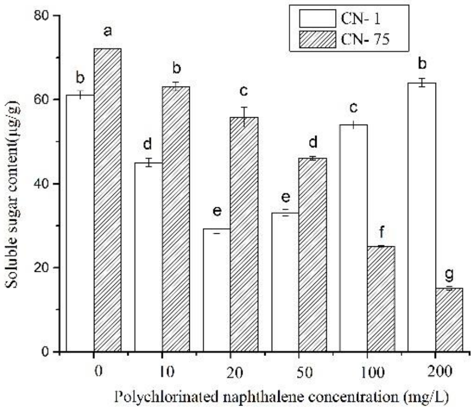

Fig. 5: Soluble sugar content with changes in PCNs concentration.

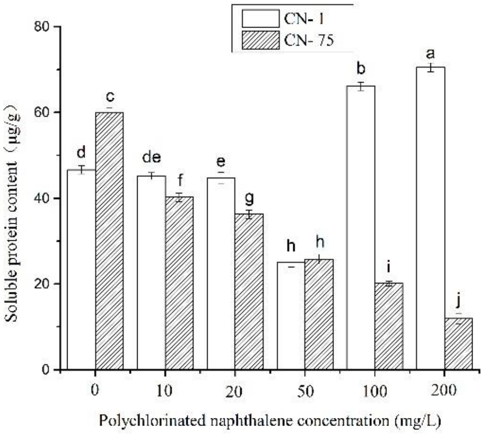

Fig. 6: Soluble protein content with changes.

CN-1 of 50 mg.L^{-1}, CN-1 of 0 mg.L^{-1} and CN-75 of 10 mg.L^{-1}, CN-1 of 10 mg.L^{-1} and CN-75 of 50 mg.L^{-1}, CN-1 of 100 mg.L^{-1} and CN-75 of 20 mg.L^{-1}, and there are significant differences in the soluble sugar content corresponding to concentrations of other indexes.

The content of soluble sugars in *Poa annua* L. decreases first and then increases with the concentration of CN-1 increasing. When the concentration is 20 mg.L^{-1}, the content of soluble sugars in *Poa annua* L. reaches the minimum, decreasing by 51.2% compared with the control group. Then, the content of soluble sugars in plants increases with the increase of concentration of CN-1. When the concentration of CN-1 is 200 mg.L^{-1}, the content of soluble sugars in plants is 6.1% higher than that in the control group.

The content of soluble sugars in *Poa annua* L decreases with the continuous increase of the concentration of CN-75. Compared with the concentration of the previous group, when the concentration of CN-75 increases accordingly, the contents of soluble sugars decrease by 12.5%, 5.3%, 24%, 44.5%, and 38.1%.

Soluble Proteins Content

The changes of soluble proteins in plants with changes in PCNs concentration are shown in Fig. 6. According to the variance analysis by SPSS software, there is no significant difference ($P > 0.05$) between the two groups with CN-1 of 10 mg.L^{-1} and CN-75 of 0 mg.L^{-1}, and there are significant differences in the soluble protein content corresponding to concentrations of other indexes.

The content of soluble proteins in plants decreases first with the increase in the concentration of CN-1. When the concentration of it reaches a certain level, the content of soluble proteins in plants increases accordingly. When the concentration of it reaches 50 mg.L^{-1}, the content of soluble proteins in *Poa annua* L. reaches the minimum, decreasing by 46% compared with the control group. When the concentration of it reaches 200 mg.L^{-1}, the content of soluble proteins reaches the maximum, increasing by 50% compared with the control group.

The content of soluble proteins decreases with the continuous increase of the concentration of CN-75 d. When the concentration of CN-75 increases accordingly, the contents of soluble proteins decrease by 32.8%, 12.3%, 26.7%, 22.0%, and 40.8%, compared with the concentration of the previous group.

MDA Content

The changes in the content of MDA in plants with changes in PCNs concentration are shown in Fig. 7. The content of MDA in plants increases then decreases with the increase of concentration of CN-1. When its concentration reaches a certain level, the content of MDA in plants reaches the maximum. When its concentration is 50 mg.L^{-1}, the content of MDA in *Poa annua* L. reaches the maximum, increasing by 289.8 % compared with the blank control group. With a continuous increase of concentration of CN-1, the content of soluble proteins decreases, but still increases compared with the blank control group.

In a normal growth environment, the content of MDA in *Poa annua* L. is only 0.03 μmol.g^{-1}. When the concentrations of CN-75 are 10 mg.L^{-1} and 20 mg.L^{-1}, the contents of MDA are 0.05 μmol.g^{-1} and 0.10 μmol.g^{-1}, respectively, showing a slight increase compared with those under normal growth conditions; When the concentration of CN-75 is 100 mg.L^{-1}, the content of MDA increases sharply to 0.40 μmol.g^{-1}. Then with a further increase in its concentration, the content of MDA decreases sharply.

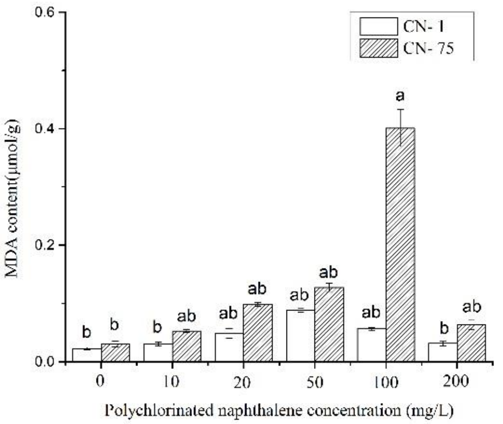

Fig. 7: MDA content with changes in PCNs concentration.

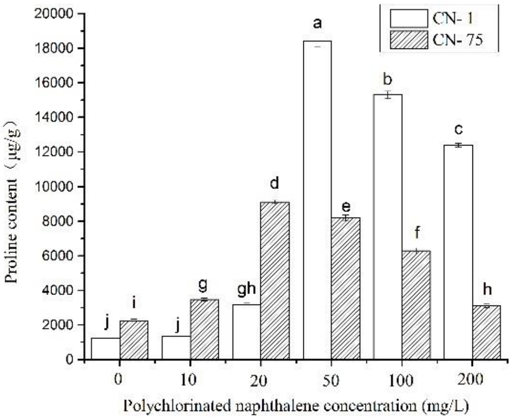

Fig. 8: Proline content with changes.

Proline Content

The changes in the content of proline in plants with changes in PCNs concentration are shown in Fig. 8. Among them, there is no significant difference ($P > 0.05$) among the CN-1 of 0 mg.L^{-1} and CN-1 of 10 mg.L^{-1}, and there are significant differences in proline content corresponding to concentrations of other indexes.

With the increase of the concentration of CN-1, the proline content in *Poa annua* L. leaves increases first and then decreases. When its content is 50 mg.L^{-1}, the proline content increases 22 times compared with the control group. At this time, the proline content in plants reaches the maximum, and the cell structure might have changed to work abnormally. When its content is 200 mg.L^{-1}, the corresponding proline content increases by nearly 10 times compared with the control group.

With the increase of the concentration of CN-75, the proline content in *Poa annua* L. leaves increases first and then decreases. When the concentration of CN-75 is 20 mg.L^{-1}, the proline content increases sharply compared with that under low concentration stress. After CN-75 content reaches 50 mg.L^{-1}, the proline content decreases.

DISCUSSION

(1) With the increase of the concentration of CN-1, the content of chlorophyll in *Poa annua* L. plants increases correspondingly. When it reaches the growth peak, the content of chlorophyll in plants will decrease with the increase of the concentration of CN-1. The above shows that CN-1 can be taken as the nutrient for the growth of *Poa annua* L. but the increased concentration is harmful to *Poa annua* L., affecting its photosynthesis. The results of this experiment showed that the content of chlorophyll a and b in *Poa annua* L. leaves increased first and then decreased with the increase of the concentration of CN-1, which is consistent with the research of Li et al. (2018) that salt stress causes the increase in the chlorophyll content in spathiphyllum with the increase of alt stress concentration.

With the increase of the concentration of CN-75, the chlorophyll in *Poa annua* L. leaves is degraded and the content decreased in case the leaves are severely damaged. While the carotenoid content starts to increase and leaves become withered and yellow. This trend is consistent with the results of the effects of naphthalene stress on the mangrove plant, *Kandelia candel*, studied by Lu et al. (2008). The PAHs concentration reaching a certain level will harm the plants and decrease the contents of chlorophyll *a*, chlorophyll *b* and total chlorophyll in plant leaves.

(2) With the increase of the concentration of CN-1, the normal metabolic activities of the cells in the plant are disturbed, causing weakened stress resistance of the cells, and decreased soluble sugar content in the plant. The concentration value of CN-1 increasing to a certain degree may stimulate plant cells badly and cause cell mutation, thus increasing the soluble sugar content in the plant.

With the continuous increase of concentration of CN-75, the soluble sugar content in *Poa annua* L. gradually decreases, indicating that the sugar in *Poa annua* L. decreases under the effect of CN-75. At the early stage of stress, due to water deficit in the plant, the amylolysis of leaves is enhanced and photosynthetic product output is slowed, causing sugar accumulation; with further strengthening of stress, water deficit is exacerbated, and the chloroplast thylakoid structure is destroyed.

At the same time, stoma opening and closing, and the photosynthetic rate of leaves are inhibited, which makes photosynthesis weakened and photosynthetic products reduced. Thus, the accumulation of soluble sugars in the leaves tends to slow down or stop. This is consistent with changes in soluble sugar and soluble protein in maize leaves under Pb (lead) stress studied by Zheng et al. (2006).

(3) With the increase of the concentration of CN-1, the content of soluble proteins in plants decreases first and then increases. Other studies have shown that the content of soluble proteins in plants growing under stress conditions will changes certainly, and based on this, the corresponding structural functions of plant cells will change, thus further influencing the regulation of plant cells (Wei 2010). Jiao et al. (2019) studied the oil-contaminated reed seedlings and found that the content of soluble proteins in the reed seedlings did not increase but was lower than that of the control group, which was consistent with the experimental study of the effect of CN-1 with a low concentration on *Poa annua* L. but was opposite to the effect of CN-1 with a high concentration on *Poa annua* L.

With the increase of concentration of CN-75, the content of soluble proteins decreases. Strong pollution stress will destroy the cell's biofilm severely and will have irreversible effects on the growth of plants. Excessive stress will inhibit the normal growth of the plant. Restrictions on plant growth and death cause a reduction in the content of the matter. The results are consistent with the changes of soluble sugars and soluble proteins in maize leaves under Pb (lead) stress studied by Zheng et al. (2006).

(4) With the increase of the concentration of CN-1, the content of MDA in plants first increases and then decreases. Chen et al. (2013) studied that the MDA content in *Bidens pilosa* leaves, under the stress of heavy metal Cd, increases first and then decreases with the increase of Cd stress concentration, which is consistent with this paper.

The MDA content in *Poa annua* L. increases first with the increase of concentration of CN-75. It increases sharply and then decreases with the concentration of CN-75 reaching 50 mg.L^{-1}. This indicates that CN-75 with low concentration will certainly damage the cell membrane of *Poa annua* L. but will not affect all life activities of the cells. The plant itself has resistance and repair functions to stress. In case the concentration of CN-75 is too high and the stress is huge, the cell membrane in the plant will be damaged to a large extent causing function failure of the membrane, and finally, the plant may die. This can be evidenced by the death of *Poa annua* L. in this experiment.

(5) The proline content in *Poa annua* L. leaves increases first and then decreases with the increase of concentration of CN-1. In the beginning, proline content in plants continues to increase. The higher the concentration of CN-1, the higher the proline content in plants. When the CN-1 content is 50 mg.L^{-1}, the proline content increases 22 times compared with that of the control group. At this time, the cell structure of the plant may have changed to work abnormally. CN-1 is harmful to the normal growth of *Poa annua* L., so to alleviate the toxic effects of CN-1, the plant will produce a large amount of proline so as to enhance the adjustment inside and outside the cell membrane. Therefore, CN-1 has strong toxic effects on the growth of plants. The changing trend of proline content in this experiment is the same as the changing trend of proline content in spathiphyllum leaves under salt stress studied by the scholar, Li et al (2018).

The proline content in *Poa annua* L. leaves increases first and then decreases with the increase of concentration of CN-75, which is consistent with the results of the effects of NaCl stress on the growth of mulberry seedlings studied by Dong et al. (2017). When the concentration of CN-75 is 20 mg.L^{-1}, the proline content increases sharply compared with that under the stress of low concentration, which shows that the cell structure and function of *Poa annua* L. are severely damaged under the stress of high concentration. The proline content decreases when the concentration of CN-75 reaches 50 mg.L^{-1}, which is related to the death of *Poa annua* L. under the stress of CN-75 of 50 mg.L^{-1}.

CONCLUSIONS

In conclusion, chlorophyll *a* and *b* in plants increase first and then decrease with the increase of CN-1. But the stress of CN-75 on plants is stronger, and the chlorophyll a and b in plants continue to decrease with the increase of CN-75. The carotenoid content in plants increases first and then decreases with the increase of concentration of CN-1, and it decreases first and then increases with the increase of concentration of CN-75. Soluble sugar and soluble protein in plants decrease first and then increase with the increase of concentration of CN-1, and continue to decrease with the increase of concentration of CN-75. This may be because CN-75 is more toxic to plants making cells lose water rapidly, and the biofilm is damaged, which is irreversible to organisms; the MDA content in plants increases first and then decreases with the increase of concentration of CN-1. When the content of it reaches 50 mg.L^{-1}, the MDA content in plants reaches the maximum. The MDA content in plants increases first and then decreases with the increase of concentration of CN-75. But when its content reaches 50 mg.L^{-1}, the MDA content increases sharply. This may be because CN-75 of low concentration damages the cell membrane certainly, but the plant has the resistance and repair functions to stress. Too high CN-75 concentration will damage the cell membrane badly, and cause membrane function failures, finally causing the death of plants. The proline content in plants increases first and then decreases with the increase of concentration of CN-1 and CN-75. The difference is that CN-1 reaches its maximum at 50 mg.L^{-1}, while CN-75 starts decreasing when reaching its maximum at 20 mg.L^{-1}. Based on the above, it can be seen that the tolerance of plants to CN-75 is not good as to CN-1.

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