Fluorine Accumulation in Sunflower and Its Photosynthetic Response

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Abstract In order to investigate the practicability to phytoremediation fluoride in wastewater by sunflower, experiment was carried out by hydroponic culture with oilseed type sunflower (Helianthus annuus L.) seedlings. The results indicated that fluorine concentrations in organs of sunflower were significantly increased with increasing solution concentrations and the content order of fluorine in sunflower organs was: roots> leaves> stems. The translocation factor was between 0.045-0.095, indicating that sunflower cannot effectively transport fluorine ions from the roots to the aerial part. The \( P_n \), \( G_s \) and \( T_r \) of sunflower leaves decreased significantly with the increase of fluoride concentration. Those results showed that the main reason for the decrease of \( P_n \) was the stomatal limitation when the \( F^- \) concentration was between 0 and 20 mg L\(^{-1}\), and the non-stomatal limitation was the main reason for the decrease of \( P_n \) when the concentration of \( F^- \) was between 50 and 150 mg L\(^{-1}\). The maximum concentration of fluoride ions in which sunflower can endure was 87 mg L\(^{-1}\).

Keywords: sunflower, fluorion, enrichment, photosynthethesis

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1. Introduction

Fluorine, which widely exists in atmosphere, soil and water, is beneficial for dental health in small amount, but also leads to severe adverse effects in high level on humans. Excessive intake of fluorine will cause great damage to human body, animals and plants [1,2]. At present, fluorine pollution is very serious in China. Due to a large amount of fluoride-containing flue gas, dust, metallurgical slag and waste water produced in the process of iron and steel smelting, the atmosphere, soil and water have been seriously polluted [3]. The chemical property of fluorine is easy to migrate with water, and the study shows that about 2/3 of fluorine in human body comes from water and 1/3 from food [4]. Therefore, it is very important to study fluorine-containing waste-water purification and its effect on animals and plants. At present, there are many kinds of methods used to purify industrial waste-water containing fluorine, mainly concentrated in physical and chemical methods, and the commonly used methods are mainly adsorption method and precipitation method [5]. In recent years, many scholars have begun to study the potential of aquatic plants to repair fluorine in water [6,7]. Many studies have shown that many plants not only have a certain degree of resistance to fluoride, and it can absorb and accumulate fluoride in the whole growing season. Therefore, it is of great practical significance to use phytoremediation to remove fluoride in water and reduce endemic fluorosis [8].

Sunflower (Helianthus annuus) is the second largest oil crop after rape in the world. It has high economic value, wide planting area, salt and alkali tolerance, drought tolerance, barren tolerance and strong adaptability and is easy reproduction [9,10]. Guo Ping found that sunflower is a heavy metal enriched plant, and its seedlings have strong enrichment ability to Pb, Cu [11]. Jing Tao [12] proved that sunflower has certain purification ability to aniline in a certain concentration range of aniline. Sunflower has a certain ability to absorb and purify heavy metals and organic matter, so it has a potential in the enrichment and remediation fluoride pollution water. In order to explore sunflower viability to purify fluoride pollution in water body, the effects of different concentrations of fluoride on photosynthetic capacity of sunflower and the enrichment of fluoride in sunflower were studied by hydroponics.

2. Materials and Methods

2.1. Design of the Experiment

The experiment was carried out in August 2016 at a plastic greenhouse in the Forestry experimental Station of...
Shandong Agricultural University. The hydroponics test was carried out with oilseed sunflower (Helianthus annuus Linn.). After 24 hours of immersion, seeds were immersed in sterilized quartz sand and irrigated with tap water. After germination, healthy seedlings of the same growth status were selected and placed in a 250mL triangle bottle filled in nutrient solution. One plant was planted in each bottle, and the culture medium was half strength Hoagland nutrient solution. The triangle bottle was wrapped in black plastic to inhibit the growth of algae. After 4 weeks of culture, the healthy plants with the same growth status were selected and treated with different concentrations of fluoride. A total of six treatments with fluoride addition were set up, which were 0, 10, 20, 50, 100, 150 mg L\(^{-1}\). After six days of treatment under the same conditions, the photosynthetic parameters of leaves were determined, then the different organs of sunflower was sampled for fluorine content determination.

2.2. Sampling and Determination Methods

2.2.1. Measurement of Photosynthetic Parameters

In sunny day, the mature leaves in the middle of each plant were selected for analysis. The photosynthetic parameters such as transpiration rate (\(T_r\)), net photosynthetic rate (\(P_n\)), stomatal conductance (\(G_s\)), intercellular carbon dioxide concentration (\(C_i\)) were determined with CIRAS-2 photosynthesis system (PP Systems, UK).

2.2.2. Sampling Method

After the termination of experiment, the roots, stems and leaves of each plant were sampled and cleaned with tap water for 2-3 times to remove surface dust and appendages. Then these samples were rinsed with deionized water for 2 times, and finally were baked in the blast dryer at 60°C-80°C for 24 hours until constant weight. After cooling, the dried sample was crushed by electric pulverizer, and the powder samples were obtained through the 1mm sieve. The samples were placed in small glass bottles and placed in the dryer.

2.2.3. Determination of Fluorine Content in Sunflower

The fluorine content in sunflower was performed with a fluoride ion-selective electrode according to a previous work [13]. Standards solutions (0.1-10 mg L\(^{-1}\)) were made from a stock solution (100 mg L\(^{-1}\)) of sodium fluoride. Firstly, 0.50 g plant sample was accurately weighed, and then it was placed in 50 mL plastic beaker. Secondly, 20 mL 0.05 N nitric acid solution was added to each sample and stirred for 12 hours. Thirdly, 20 mL 0.1 N potassium hydroxide solution was added and stirred for 20min. After that, 10 mL supernatant was added to a 50 mL plastic volumetric flask, then 20 ml total ionic strength adjustment buffer (TISAB) was added before fluoride estimation. Finally, the samples were measured by fluoride ion selective electrode.

Transfer coefficient

\[
\text{Transfer coefficient} = \frac{\text{mean fluorine content above the ground part} (\text{mg kg}^{-1})}{\text{mean fluorine content at the root} (\text{mg kg}^{-1})}
\]

Enrichment coefficient

\[
\text{Enrichment coefficient} = \frac{\text{root or above ground fluorine content} (\text{mg kg}^{-1})}{\text{fluorine content} (\text{mg L}^{-1}) \text{ in water}}
\]

2.2.4. Data Process

SPSS 17.0 and Excel 2010 software were used for data analysis and graph. The difference between control and treatments were analyzed by one-way analysis of variance (AVOVA) and Duncan’s multiple-range test was used for multiple comparisons.

3. Results

3.1. Accumulation of Fluorine in Organs of Sunflower

The fluorine content in organs of sunflower increased with the increase of fluoride concentration in culture medium (Figure 1). The content of fluorine in root and leaf was monotonously increasing, and the maximum of fluorine content was obtained in 150 mg L\(^{-1}\) fluoride hydroponics. When the fluoride concentration was equal or greater than 50 mg L\(^{-1}\), there was significant difference of accumulation of fluorine in roots and leaves between the treatment group and the control (P<0.05). The fluorine concentration in roots was 53.23 times of that in the control, while that in leaves and stems was 14.15 and 8.85 times of that in the control, indicating that fluorine absorption by plant mostly stored in the roots. The order of fluorine concentration in organs of sunflower was root > leaf > stem.

3.2. Enrichment Coefficient and Fluorine Transfer Coefficient of Sunflower

As shown in Table 1, the enrichment coefficient of root and above ground part decreased monotonously with the increase of fluoride concentration. The enrichment coefficient of the roots was significantly higher than that of the above ground parts, indicating that the roots were the main enrichment organs of fluorine. The transfer coefficient decreased first and then increased with the change of fluoride concentration and reached the lowest value in 50 mg L\(^{-1}\) treatment and the highest in 150 mg L\(^{-1}\) treatment, which indicated that the mechanism of fluorine transfer from root to above ground part of sunflower might be different under the condition of low fluoride concentration and high fluoride concentration. The transfer coefficient was less than 0.1, which indicated that sunflower could not effectively transfer fluorine from the root to the above ground part.
Table 1. Enrichment coefficient and transfer factor of fluorine in sunflower

| Concentration (mg L⁻¹) | Root Enrichment coefficient | Above ground Enrichment coefficient | Transfer factor |
|------------------------|-----------------------------|-------------------------------------|-----------------|
|                        | [μmol (m²·s⁻¹)] (%)         | [μmol (m²·s⁻¹)] (%)                 | [μmol (m²·s⁻¹)] (%) |
| 10                     | 10.52±0.143 a                | 1.67±0.046 a                        | 0.0793 b        |
| 20                     | 6.50±0.144 b                 | 1.02±0.049 b                        | 0.0788 b        |
| 50                     | 6.24±0.034 b                 | 0.56±0.012 c                        | 0.0450 d        |
| 100                    | 3.51±0.012 c                 | 0.49±0.018 c                        | 0.0694 c        |
| 150                    | 2.93±0.023 d                 | 0.55±0.022 c                        | 0.0948 a        |

Different lowercase letters showed significant difference from each other (p<0.05).

Table 2. Effects of different concentration of fluoride on photosynthesis of sunflower

| Concentration (mg L⁻¹) | Net photosynthetic rate (Pn) [μmol (m²·s⁻¹)] (%) | Stomatal conductance (Gs) [μmol (m²·s⁻¹)] (%) | Transpiration rate (Tr) [μmol (m²·s⁻¹)] (%) | Intercellular CO₂ concentration (Ci) (μL L⁻¹ (%) |
|------------------------|--------------------------------------------------|-----------------------------------------------|---------------------------------------------|-----------------------------------------------|
| 0                      | 15.22±0.19 a                                    | 179.58±7.14 a                                | 2.80±0.06 a                                 | 218.83±2.21 b                                |
| 10                     | 13.31±0.01 b                                    | 151.42±2.23 b                                | 2.24±0.15 b                                 | 207.75±1.70 b                                |
| 20                     | 11.95±0.15 c                                    | 123.08±3.95 c                                | 2.11±0.11 b                                 | 191.50±2.84 e                                |
| 50                     | 9.76±0.18 d                                     | 118.17±3.53 c                                | 1.83±0.04 c                                 | 229.33±1.10 b                                |
| 100                    | 7.21±0.21 c                                     | 84.50±3.27 c                                 | 1.48±0.07 d                                 | 230.92±0.74 b                                |
| 150                    | 4.40±0.21 f                                     | 71.42±2.97 c                                 | 1.19±0.11 e                                 | 272.58±3.25 a                                |

Different lowercase letters showed significant difference from each other (p<0.05).
3.3. Effect of Fluoride Treatment on Photosynthetic Parameters of Sunflower

As shown in Table 2, \( P_n \), \( G \), and \( T \), of sunflower leaves decreased gradually with the increase of fluoride concentration, and there was a significant difference compared with the control (\( P<0.05 \)). When fluoride concentration reached 150 mg L\(^{-1} \), \( P_n \), \( G \), and \( T \) were only 28.9\%, 40\% and 42.5\% of that of control respectively. The results showed that the fluoride treatment could inhibit the photosynthesis of sunflower, and with the increase of fluoride concentration, the inhibition effect was more serious. However, the \( C_i \) decreased at first and then increased with the increase of fluorine concentration, and reached the minimum when fluoride concentration was 20mg L\(^{-1}\),and reached the maximum value when fluoride concentration was 150mg L\(^{-1}\).

4. Discussion

4.1. Enrichment of Fluorine by Sunflower

Different plants have different fluorine enrichment capabilities. Yuan et al. reported that the enrichment capability of fluorine in different parts of rapeseed plant was root > leaf > stem [14]. Jha et al. found that the distribution of fluorine in spinach was root > stem > leaf [15]. Qu et al. reported that there were two situations of distribution of fluorine content in the seedlings: when the concentration of fluoride ion in the culture medium is 0.5-5mmol L\(^{-1}\), the order of fluorine content in \( Pterocarya stenoptera \) seedlings was root > leaf > stem, when the concentration of fluorine ion was 10mmol L\(^{-1}\), it was root > stem > leaf [8]. In our experiment, the order of fluorine content in organs of sunflower was root > leaf > stem.

The main organ of the enrichment of fluorine in sunflower is root, but the amount transferred to stem and leaf is less, which indicates that the root has the function of interception during the whole absorption process, which is helpful to protect other organs of the plant from being poisoned by excessive fluorine. In this experiment, the fluoride mainly enter the sunflower through the root , and the stem is the channel of transport element, so the accumulation of fluorine in stem is the least. The enrichment coefficient can indicate the ability of plants to absorb elements. The results show that the enrichment coefficient of roots and above ground parts decreased with the increase of fluoride concentration, which indicates that the absorption of fluorine by sunflower is mainly controlled by its own demand. The transfer coefficient decreased firstly and then increased with the increase of fluoride concentration, and reached the highest in 150 mg L\(^{-1}\) treatment, 0.0948, but also less than 0.1, which indicated that the transfer ability of fluorine to sunflower was weak. Li et al. reported that tea seedlings were root > leaf > stem when treated with 150mg L\(^{-1}\) fluoride, and the accumulation of fluorine in the roots, stem and leaves of tea seedlings was 2492.78, 674.63 and 3646.85 mg kg\(^{-1}\) respectively [17]. While in this experiment, when sunflower grew in 150mg L\(^{-1}\) for 14 days, the accumulation of fluorine in the root, stem and leaf of sunflower was 438.76, 18.67 and 64.5 mg kg\(^{-1}\), respectively, which showed that the enrichment ability of sunflower to fluorine was much smaller than that of tea plant. Sunflower also had some ability to enrich other heavy metals.

4.2. Effect of Fluoride Photosynthesis of Sunflower

The damage caused by fluoride pollution to plants is mainly divided into visible injury and non-visible injury. Visible injury includes leaf chlorosis, leaf necrosis, leaf shedding, growth inhibition and so on, while non-visible injury is mainly caused damage to photosynthetic structure and cell structure of plant, respiratory metabolism and the effects of genetic material [18,19]. Li et al. reported that \( P_n \) of tea seedlings decreased to 30.8\% of the control group when tea seedlings were treated with 10 mg L\(^{-1}\) fluoride for 30 days [20], while in our experiment, when the sunflowers were treated with 10 mg L\(^{-1}\) for 6 days, the \( P_n \) decreased to 87.5\% of the control group, which showed that the inhibitory effect of fluorion on photosynthesis of tea plants was greater than that of sunflowers. This may be attributed to higher accumulation of fluorine of tea seedlings than sunflower seedlings. Data in Table 2. showed that the \( P_n \) of sunflower decreased to 47.4\% of the control when F\(^{-}\) concentration was 100 mg L\(^{-1}\), which was lower than that of 50\% under normal condition, and plant could not survive in a long time. The relationship between the concentration of fluorine ion and the percentage of the decrease of \( P_n \) was well simulated by the quadratic curve, \( X_2=-2.2423+0.792345*X_1+0.019534*X_1^2 \). Among them, \( X_2 \) is the concentration of fluorine ion and \( X_1 \) is the percentage of the decrease of photosynthetic rate. It is calculated that the concentration of fluorine ion is 87.3 mg L\(^{-1}\) when the percentage of the photosynthetic rate is 50\% of the control. Its is suggested that sunflower was only suitable for treating wastewater with fluoride concentration lower than 87.3mg L\(^{-1}\).

There are many factors to decrease photosynthetic rate, they are divided into two categories: stomatal limitation and non-stomatal limitation. The non-stomatal restriction is due to the increase of stomatal diffusion impedance, the decrease of CO\(_2\) solubility, the decrease of rubisco CO\(_2\) affinity and the decrease of regeneration ability of ribulose 1,5-diphosphate in mesophyll cells. Fluorine accumulated in leaf inhibited the activity of RuBP carboxylase. The stomatal restriction was due to the decrease of stomatal opening of mesophyll cells, which resulted in the decrease of \( C_i \) and blocked the supply of CO\(_2\) in chloroplast [21]. Data in Table 2. shows that fluoride treatment has obvious inhibitory effect on the photosynthesis of sunflower. At the same time, it was found that the concentration of intercellular carbon dioxide was not increasing monotonously, but decreasing first and then increasing. The \( P_n \), \( G \), and \( C_i \) of leaves decreased at 0~20mg L\(^{-1}\). Therefore, the main reason for the decrease of \( P_n \) was stomatal limitation when fluorine concentration is ≤20mg L\(^{-1}\). When fluorine concentration is >20~150 mg L\(^{-1}\), \( P_n \) and \( G \) decreased, while the concentration of \( C_i \) increased. In this case, the main reason for the decrease of \( P_n \) was due to the non-stomatal limitation.
5. Conclusion

To sum up, sunflower has certain enrichment ability to fluorine. With the increase of fluoride concentration in culture medium, the enrichment amount of fluorine in sunflower increased gradually, and $P_n$ decreased gradually. Sunflower was only suitable for treating wastewater with fluoride concentration lower than 87.3mg L$^{-1}$.

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