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Physiological Responses of *Pistia stratiotes* and Its Fluoride Removal Efficiency

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Abstract: Phytoremediation technology using aquatic plants is being used increasingly in constructed wetlands to purify wastewater. The physiological responses of water lettuce (*Pistia stratiotes*) and its effectiveness in removing fluoride (F^-) from water are described in this article. The results indicate that *Pistia stratiotes* has the ability to accumulate F^-. The removal efficiency ranged from 27.79% to 56.32% for the various initial F^- concentrations tested, and was highest (56.32%) in the highest initial concentration group 60mg/L. The F^- concentrations in control groups (without *Pistia stratiotes*) changed very little, from –1.135% to –0.007% of the initial concentrations. At the highest removal rate, the bioconcentration factor was 7.84. The rate of purification conformed to the Michaelis-Menten equation, and the correlation coefficients (R^2) were all greater than 0.97. The Specific Growth Rates (SGR) of the treatment groups were –8.03% to –1.22%, and the SGR of plants under F^- stress decreased during the experimental period. The partial correlation analysis showed that concentrations of F^- in water were strongly linearly correlated with peroxidase.

Key words: *P. stratiotes*; antioxidant enzyme; fluoride; dynamic; POD

1 Introduction

The World Health Organization (WHO) classifies fluoride as a contaminant of water for human consumption. Several industries commonly discharge wastewater containing high concentrations of fluoride, including aluminum smelters, semiconductor manufacturing, the production and use of fertilizers, glass, brick and ceramic production, and the combustion of fossil fuels (Jelenko and Pokorny, 2010; Bhatnagar et al., 2011). Excess concentrations of fluoride can cause various diseases in humans, such as osteoporosis, masculine infertility, brain damage and cancer (Harrison et al., 2005; Mahua et al., 2007). Several treatment processes have been applied to remove fluoride from aqueous solutions, such as adsorption (Medellin-Castilloa et al., 2014), reverse osmosis (Trikha and Sharma, 2014), ion exchange (Meenakshi and Viswanathan, 2007), and chemical precipitation (Huang and Liu, 1999; Guo and Tian, 2013). However, most of these processes are costly (installation and maintenance), result in secondary pollution, and require complex methods. An emerging technology in the de-fluoridation of water is phytoremediation, which is a low-cost, environmentally friendly method (Zhou et al., 2012). Plants that accumulate high concentrations of F^- and show tolerance and resistance to F^- toxicity have the potential to provide a remediation solution (Baunthiyal and Sharma, 2014).

Aquatic flora plays an important role in the phytoremediation of polluted surface waters. *Pistia stratiotes*, a free-floating aquatic macrophyte, has been studied recently due to its rapid growth in contaminated water bodies (Sinha et al., 2005). In addition to providing food and dissolved oxygen, *P. stratiotes* can trap excessive nutrients and detoxify water (Lu et al., 2010; Owamah et al., 2014). This species has been widely reported as a good bio-indicator of

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pollution and is noted for its high capacity for removing and adsorbing pollutants from aqueous solutions (Sánchez-Galván et al., 2013).

In addition to demonstrating the high removal efficiency of pollutants from aqueous solution, understanding the mechanisms of tolerance to those pollutants, especially the antioxidative defense mechanisms, is important for its use in practical application (Upadhyay and Panda, 2009). The effectiveness of *P. stratiotes* in removing F^- was evaluated here, and the correlations between water quality indices and physiological properties were also studied to understand the tolerance mechanisms.

2 Materials and methods

2.1 Plants and chemicals

Individual *P. stratiotes* plants of similar sizes were obtained from outdoor ponds in the suburbs of Xichang City, China. The plants were thoroughly washed with tap water and cleaned with deionized water prior to their use in the experiments. They were then acclimatized in plastic basins with tap water indoors for seven days. Healthy *P. stratiotes* with abundant roots were chosen and transplanted into 18 plastic containers (diameter is 25.3 cm, height is 15.1 cm, and volume is 6.8 L) with each container including with about 100–110 g biomass. The indoor air temperature was maintained between 27 ℃ and 30 ℃ and the water temperature in the plastic containers was kept between 23 ℃ and 27 ℃. All chemicals used were of analytical grade.

2.2 Chemical analyses

F^- concentrations in the aqueous solutions were measured using Fluorine reagents spectrophotometry (HJ 488—2009, China). Fluoride in plant leaves was determined by Micro-diffusion methods (Xiang and Gao, 1980). Superoxide dismutase (SOD) activity was assayed according to the method described by Beauchamp and Fridovich (1971). Catalase (CAT) activity was measured according to the method described by Aebi (1974). Peroxidase (POD) activity was measured according to Kraus and Fletcher (1994).

2.3 Experimental design

Plants in the treatment groups were exposed to initial F^- concentrations of 5, 10, 20, 40 and 60 mg L^{-1} by adding the appropriate amounts of sodium fluoride (NaF) and thoroughly mixing, and these samples were labeled AI, AII, AIII, AIV and AV, respectively. One control group of plants was cultivated with tap water and marked BI. Five control aqueous solutions without plants were prepared with F^- concentrations of 5, 10, 20, 40 and 60 mg L^{-1}, and were designated CI, CII, CIII, CIV and CV, respectively. Aqueous F^- concentrations, levels of F^- in the plants and physiological properties of the plants (SOD, CAT and POD) were measured every two days. The testing period lasted for eight days. According to our previous study, some *P. stratiotes* withered or died within eight days under the stress of high fluoride concentrations, so eight days was chosen as the duration of this study.

2.4 Data processing

The F^- uptake rate was determined using the Michaelis-Menten equation (Youngdahl et al., 1982; Song et al., 2011):

$$v = \frac{V_{\max} \times (C_0 - C)}{K_m + (C_0 - C)} \tag{1}$$

where v is the rate of uptake, mg L^{-1} h^{-1}; $V_{\max}$ is the maximum rate of uptake, mg L^{-1} h^{-1}; K_m is the Michaelis–Menten constant; C_0 is the initial concentration, mg L^{-1}; C is the concentration at time t and $T_{1/2}$ was defined as the time to halve the F^- concentration in the aqueous solution.

Total importance value of plant productivity (S) was calculated as (Ma, 2007):

$$S=Q\times R_x \tag{2}$$

where Q is the Influence value, which was assigned by the influencing power of the three indexes (wilting degree, stem growth, and lodging state) as values of 10, 10 and 5, respectively. Each influence value is divided into three ranks (R_x: 1, 1/2, and 1/5) according to severity level. A plant without any symptoms was ranked as 1, and the value was then decreased to 1/2 or 1/5 as symptoms became more severe.

3 Results and discussion

3.1 Removal efficiency of *P. stratiotes* for F^- in aqueous solution

The F^- removal efficiencies for the different initial concentrations are shown in Fig. 1.

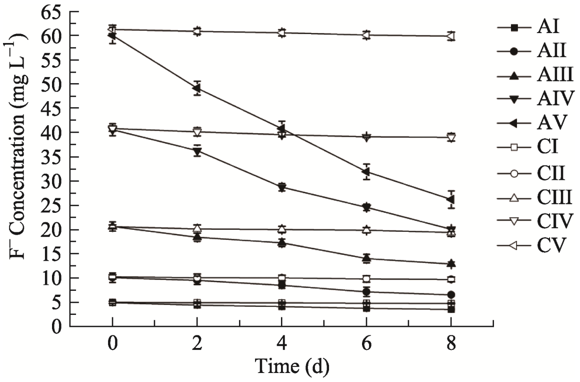

Fig. 1 Variation of F^- concentrations over time starting from different initial F^- concentrations for treatment groups (AI-AV) and control groups without plants (CI-CV).

Note: Error bars represent one standard deviation from the mean, n = 3.

The removal efficiency ranged from 27.79% to 56.32% for each of the different initial concentrations. The removal rate was highest (56.32%) in the highest initial concentration group. The F^- concentrations in the control groups changed very little, only by–1.135% to –0.007% of the initial concentrations. The initial concentrations had only a minor effect on the removal efficiency of control groups, indicating that the plants played an important role in F^- removal. The kinetic parameters of the modified Michaelis-Menten equation for F^- removal are shown in Table 1.

Table 1 Kinetic parameters of fluoride removal from treated water

Group	C_0 (mg L^{-1})	V_{max} (mg L^{-1} h^{-1})	K_m (mg L^{-1})	R^2
AI	5	0.2533	−3.4237	0.9806
AII	10	0.485	−7.1748	0.9808
AIII	20	1.1145	−16.4028	0.9766
AIV	40	2.96	−43.6132	0.9895
AV	60	5.427	−77.0471	0.9898

Data from the Michaelis-Menten equation calculations showed high correlations for the treated groups, with all correlation coefficients (R^2) greater than 0.97. This suggests that the transport of fluoride is an active process, requiring an energy supply and selective binding sites (Mohammed et al., 2002). The Michaelis–Menten constant (K_m) decreased gradually when C_0 increased from 5 to 60 mg L^{-1}. The maximum rate of uptake (V_{max}) increased with initial concentrations, indicating that the plant can take up F^- effectively under high stress. In the experiments, F^- uptake occurred through the root system and the absorbed F^- became concentrated in the leaves, which are the site of greatest evaporation Sequestration of the anion in cell vacuoles may be one possible mechanism of F^- tolerance (Santos-Diaz and Zamora-Prdraza, 2010).

3.2 Plant characteristics and growth

The growth status of the plants influences their resistance to pollutants, which is one important factor for the selection of wetland plants to use in phytoremediation. During the early stage of the experiment, all plant biomass increased, root systems became stouter and leaf areas enlarged. However, during the experimental time period, withering and lodging appeared and were more obvious for plants exposed to high concentrations of F^-. Plants in the control groups (BI) showed normal growth without any pathology. At the end of the 8-day experiment, the root systems appeared to be shorter and were clearly dying (see Table 2).

Table 2 Effect of different concentrations of fluoride on the growth characteristics of *P. stratiotes*

Treatment groups		AI	AII	AIII	AIV	AV	BI
Fresh weight (g)	Initial	101.4	101.8	101.9	101.5	103.0	102.8
	Ending	92.0	86.9	76.3	65.6	54.2	143.6
SGR (% d^{-1})		−1.22	−1.98	−3.62	−5.46	−8.03	4.18

Note: Specific Growth Rate (SGR) = $(\ln W_t - \ln W_0)/t \times 100\%$. W_t is the weight of *P. stratiotes* at *t* days, g; W_0 is the initial weight of *P. stratiotes*, g.

Based on vegetative power and the overall importance values, the groups were ranked as follows: AI (100) >AII (97.5) >AIII (81.5) >AIV (78.5) >AV (69.5). Therefore, *P. stratiotes* growth was influenced by the initial concentrations of F^-. The Specific Growth Rate (SGR) was calculated based on the initial and final biomass weights, and the SGR of treatment groups varied from −1.22% to 8.03%. The SGR of plants under F^- stress decreased during the experimental period. Presumably, harmful oxygen species were activated which could not be detoxified, and they contributed to the occurrence of visible injuries (Fornasiero, 2001; Muppala and Meenakshj, 2008). In the two highest concentration treatments (AIV, AV), some leaves of *P. stratiotes* showed discoloration on the tips, which turned tan-yellow and then faded and became desiccated.

3.3 Effect of F^-concentration in the solution on fluoride concentrations in the plants

Fluoride concentrations in *P. stratiotes* leaves increased significantly in the high F^- treatments (Fig. 2).

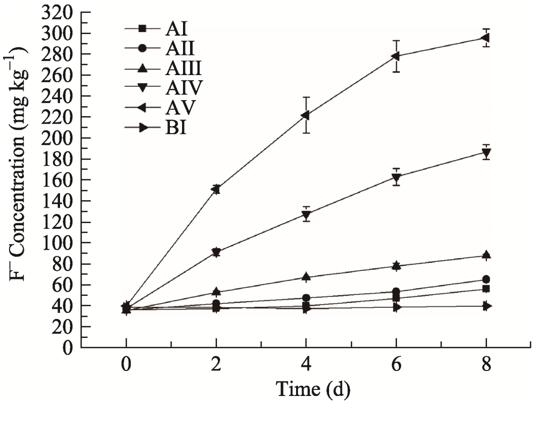

Fig. 2 Effect of initial F^- concentration on fluoride concentration in *P. stratiotes* leaves.

Note: Error bars represent one standard deviation from the mean, $n = 3$ (for treatments with F^- concentrations of 0, 5, 10, 20, 40 and 60 mg L^{-1})

The F^- concentration for control group BI was stable throughout the 8-day treatment. It has been hypothesized that when the F^- ionic strength of a solution increases, it suppresses the negative charge of the Donnan free space which allows a higher activity of F^- close to the root uptake sites, promoting F^- uptake (Stevens et al., 2010). Bioconcentration factors can be used to compare the mechanisms of element uptake and translocation in plants. The bioconcentration factors measured here were between 1.5 and 7.84

for treatment groups AI to AV. The values of this index were all higher than 1, indicating the high effectiveness of the plant in concentrating F^- from water into the *P. stratiotes* biomass with limited damage to the plants (Carolina and Raúl, 2014).

3.4 Effects of different concentrations of F^- on the activities of SOD, CAT and POD in leaves of *P. stratiotes*

Plants possess several antioxidant systems that protect them from oxidative damage. These defense systems are composed of enzymatic scavengers of activated oxygen, such as SOD, CAT and POD (Li et al., 2013). Variations in the activities of SOD, CAT and POD in the leaves of *P. stratiotes* are shown in Fig. 3, Fig. 4 and Fig. 5.

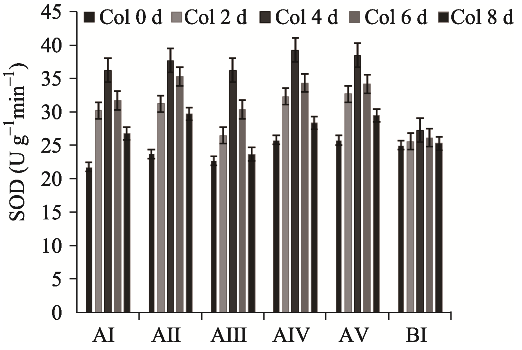

Fig. 3 Effects of different concentrations of F^- on the activities of SOD in *P. stratiotes* (treated with F^- concentrations of 0, 5, 10, 20, 40 and 60 mg L^{-1})

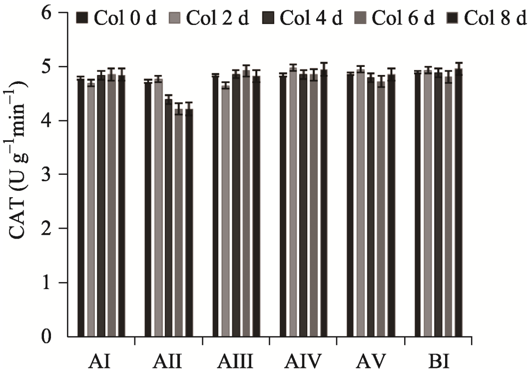

Fig. 4 Effects of different concentrations of F^- on the activities of CAT in *P. stratiotes* (treated with F^- concentration of 0, 5, 10, 20, 40 and 60 mg L^{-1})

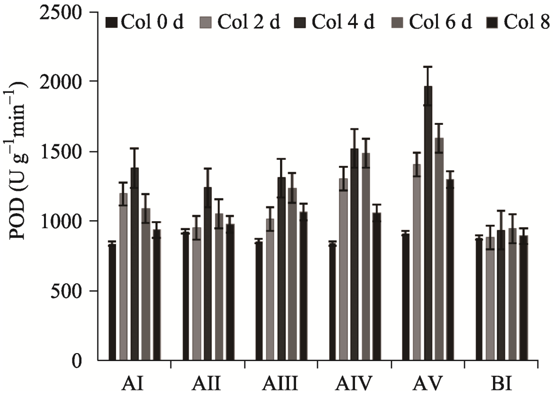

Fig. 5 Effects of different concentrations of F^- on the activities of POD in *P. stratiotes* (treated with F^- concentration of 0, 5, 10, 20, 40 and 60 mg L^{-1})

SOD is a protective antioxidant enzyme located in various cell compartments which catalyzes excess O_2^- to H_2O_2 and O_2 molecules (Sinha et al., 2009). POD can remove low concentrations of H_2O_2 from the plant. All SOD and POD activities in the leaves of *P. stratiotes* showed a similar trend of increasing at first, but then decreasing and reaching the highest concentration after four days (Fig. 3 and Fig. 4). This pattern is attributed to a decrease in adaptability at higher concentrations due to the environmental stress. When stress is increased or prolonged, the defense systems of plants will weaken as the tolerance limit is exceeded (Tewari et al., 2006). CAT is important in the removal of the H_2O_2 that is generated in peroxisomes by oxidases involved in the β-oxidation of fatty acids, photorespiration and purine catabolism (Gill and Tuteja, 2010). In this experiment, CAT fluctuated only slightly (Fig. 5) during the eight-day treatments, indicating that CAT played a minor role in stress resistance.

3.5 Partial correlation analysis between fluoride and the physiological indexes of the plant

In the multivariate correlation analysis, simple correlation coefficients may not be able to accurately reflect the correlations between the variables because such relationships are very complex and may be simultaneously influenced by more than one variable (Guo et al., 2014; Jha et al., 2015). Therefore, partial correlation coefficients (PCCs) are a better choice in this case. PCCs were used to characterize the strength of interaction between fluoride and each of the physiological indexes of the plant (Table 3).

The results revealed highly significant differences (P<0.01) between the F^- of treatment groups AI, AII, AIII, AIV; and a significant difference (0.01< P< 0.05) in the F^- in water of treatment group AV. The linear correlation between F^- in water and fluoride in leaves is high (PCCs> 0.804) for all antioxidant enzyme controls. This indicates that the removal of F^- from water is mainly due to uptake by the plant, despite the initial F^- concentration and the antioxidant enzymes. Concentrations of F^- in water were highly linearly correlated with POD (PCCs> 0.7), which demonstrates that the main enzyme reaction in *P. stratiotes* leaves under F^- stress was peroxidase.

4 Conclusions

High initial concentrations of F^- in water lead to increased plant absorption. The presence of *P. stratiotes* significantly improved the water quality by removing F^-. The rates for F^-

Table 3 Significance and PCCs between fluoride and the physiological indexes of the plant

Variable	Treatment groups		AI	AII	AIII	AIV	AV
	Significance coefficient *P*		0.0057	0.0097	0.0095	0.0012	0.0167
PCCs	Control SOD&CAT	F^- in water / Fluoride in leaves	0.999	1.000	0.804	0.989	0.999
	Control SOD&POD		0.982	0.967	0.995	0.995	1.000
	Control CAT&POD		1.000	1.000	0.987	0.993	0.997
	Control SOD&CAT	F^- in water / POD	0.900	0.848	0.999	0.781	0.706
	Control SOD&POD	F^- in water / CAT	0.653	0.645	0.990	0.486	0.958
	Control CAT&POD	F^- in water / SOD	0.911	0.804	1.000	0.855	0.672

Notes: *P*: Significance coefficient; $0.01< P < 0.05$: significant difference; $P < 0.01$: highly significant difference; PCCs: 0–0.19: very low correlation; 0.2–0.39: low correlation; 0.4–0.69: medium correlation; 0.7–0.89: high correlation; 0.9–1.0: very high correlation.

removal from the water were described well by the Michaelis-Menten equation, with correlation coefficients greater than 0.97 in all cases. The enzyme activities of SOD and POD first increased and then decreased throughout the experiment. CAT varied little across the 8-day treatments. Partial correlation analysis between the fluoride index and SOD, POD and CAT showed that POD plays an important role in the stress-resistance system (PCCs> 0.7, $P< 0.05$).

Since *P. stratiotes* is an invasive species, it is important to note that it grows easily in diverse tropical aquatic ecosystems and wastewater. As such, its growth should be strictly confined to remediation systems to avoid any unnecessary damage to native ecosystems. Further research should explore the changes in plant characteristics under high initial concentrations and longer treatment times to clarify the mechanism of F^- removal by *P. stratiotes*. The effect of initial planting density on the growth of *P. stratiotes* may be another important point for further study. The stability in aqueous media and the reusability of *P. stratiotes* need to be characterized before this system can be applied to wastewater treatment.

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大薸对水中氟化物的去除及生理响应

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摘　要：采用水生植物构建的人工湿地生态修复技术在用于净化废水方面正逐渐兴起。试验采用大薸为实验材料，采用室内培养方法，研究该湿地植物对水中氟化物水体的去污效果及其植物生理响应。实验结果表明大薸可富集水中氟化物，对于所试验的水中氟化物初始浓度，去除率在27.79% 到56.32%之间，最高的去除效率出现在氟化物最高初始浓度60 mg L^{-1}时。对照组中氟化物的浓度波动很小，最终浓度为其对应初始浓度的−1.135% – −0.007%，处理组净化速率符合Michaelis-Menten方程，其相关系数均高于0.97。在试验周期内，大薸在氟化物的胁迫下比生长速率（SGR）呈降低趋势，处理组植物的SGR在−8.03%与−1.22%之间。对照组植物的SGR呈增长趋势，为4.18%。偏相关分析表明水中氟化物浓度与POD呈高度线性相关。

关键词：*P. stratiotes*；抗氧化酶；氟化物；动力学；POD