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Removal of Fluoride from Water by Five Submerged Plants

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Abstract Studies were conducted on the bioconcentration of fluoride (F^-) in five submerged plants species. *Ceratophyllum demersum, Hydrilla verticillata, Potamogeton malaianus*, *Myriophyllum verticillatum* and *Elodea nuttallii* were all able to remove F^- from water to some degree of efficiencies. At 5–20 mg F^-/L culture solution, *C. demersum* had the best F^--removal performance, *E. nuttallii* had the poorest F^--removal performance among these plants. The relative growth rate (RGR) of the five species varied in different concentrations of F^-, of which *C. demersum* had the highest RGR. Its RGR decreased by 26.3 %, 63.2 % and 73.7 % from controls at 5, 10 and 20 mg F/L, respectively.

Keywords Fluoride · Submerged plant · Removal · Relative growth rate

Fluoride (F^-) is a common ion associated with salts found in most soils and rocks. It dissolves in water, and may contaminate water bodies during rainfall. Water acts as predominant source of F^- to cause fluorosisin in the endemic areas (Jha et al. 2008). Fluoride has been considered both as an essential element and potent environment pollutant at high concentrations causing a number of disorders (fluorosis) among the consumers. Fluorosis in general, has been identified in various countries (Sinha et al. 2000). Almost half of the rural areas of Guizhou province and many regions within the 11 adjacent provinces in southwestern China have a long history (at least 70 years) of endemic fluorosis, including dental fluorosis and osteofluorosis along with its associated deformities and disabilities (Liang et al. 2011). Unless effectively controlled, industrial F^- emissions, especially from phosphate fertilizer plants and plants producing aluminum, iron, glass, and ceramics, are significant environmental hazard to the biocenosis of water ecosystems (Maria et al. 2001).

The incidence of dental fluorosis is caused by excess F^- consumption. Stevens et al. found that the ionic species of F^- in solution had a marked influence on the uptake of F^- by plant roots with complex species being more readily taken up by the roots than the free F^- ions (Stevens et al. 1997; Stevens et al.1998).

Phytoremediation is an expanding technology that employs higher plants for the cleanup of contaminated environments. A major advantage that it has over physical remediation methods is its lower cost (Santos-Diaz and Zamora-Prdraza 2010). F^- may adversely affect aquatic vegetation, causing stunting of growth or even death (Shrike and Chandra 1991). The toxic action of F^- on plants is manifested chiefly by its effect on primary physiological functions such as photosynthesis or metabolic cycles. Moreover, in certain instances growth-stimulating effects of F^- on some plants have been observed (Joy and Balakrishnan 1990).

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Because of environmental concerns, the present study was undertaken to explore the phytoremediation of high-fluoride drinking water. Different species of submerged plants (*Ceratophyllum demersum, Hydrilla verticillata, Myriophyllum verticillatum, Potamogeton malaianus, Elodea nuttallii*) were cultured in different F^- concentrations (0, 5, 10, 20 mg F^-/L) in the same time. We evaluated their abilities to accumulate F^- while removing it from water, as well as the effect of F^- on their growth.

Materials and Methods

The submerged plants of *C. demersum, H.verticillata, M. verticillatum, P. malaianus* and E. nuttallii were collected from unpolluted rivers in Zhengzhou (34°16′–34°58′N, 112°42′–114°14′E), China. Samples were rinsed to remove invertebrate grazers and then acclimatized in 10 % Hoagland's solution for 1 month in the laboratory campus. Sturdy plants (approximately 20 cm in length) were cut off from acclimatized mother plants and care was taken to use the plants with almost the same biomass (30.0 g wet weight). These plants were transplanted to the clear 5 L culture plastic buckets (diameter 17.5 cm, height 26.6 cm, volume 6.8 L).

The experiment was carried out in a greenhouse in September, with controlled temperature and illumination. The mean water temperature was 22 ± 2°C. The illumination intensity was 122 μmol m^{-2} s^{-1}/dark 14/10 h. Nutrient solution (5 L) was prepared in cylindrical plastic buckets, with 4–5 cm of quartz sand spread out on the bottom. A thin black low-pressure polyethylene membrane was wrapped around the buckets. Each microcosm was planted with 30 g (wet weight) submerged plants. The culture solutions were prepared using deionized water (Table 1). A stock solution was prepared by dissolving 442 mg NaF in 2 L deionized water (100 mg F^-/L). Appropriate dilutions were made to obtain 5, 10 and 20 mg F^-/L culture solutions in 10 % Hoagland's solution, and the concentration gradients were set based upon the fluoride proportion settings of Chinese high-fluoride drinking water. Experimental controls consisted of 30 g (wet weight) submerged plants and 10 % Hoagland's solution. The pH value was set as seven referring to normal water. Each group was repeated three times, and altogether 69 culture plastic buckets were used, including blank controls with no plants. The pH in each culture plastic buckets was adjusted two times each day with NaOH and HCl solutions. The culture solutions were replaced every 3 days to keep nutritive elements constant.

The experimental period was 32 days. Samples of culture solution (3 mL) were collected from water surface of the buckets on 2, 4, 8, 16 and 32 days, Samples were immediately filtered through whatman No. 42 filter paper and kept at 0–4°C until analyzed within 24 h. F^- concentration was measured using an ion chromatography (ICS–90, Dionex, California, USA) with F^- solutions of known concentration for calibration. NaF was used for the preparation of standard solutions. pH values of water samples were determined with pH meter (Mettler-Toledo 320–S, Zurich, Switzerland). The RGR results were analyzed statistically by SPSS (Version 10.0). Two-way ANOVA was performed to test the effects of different submerged species in different F^- concentrations on RGR. The multiple comparisons used Duncan's new multiple range test.

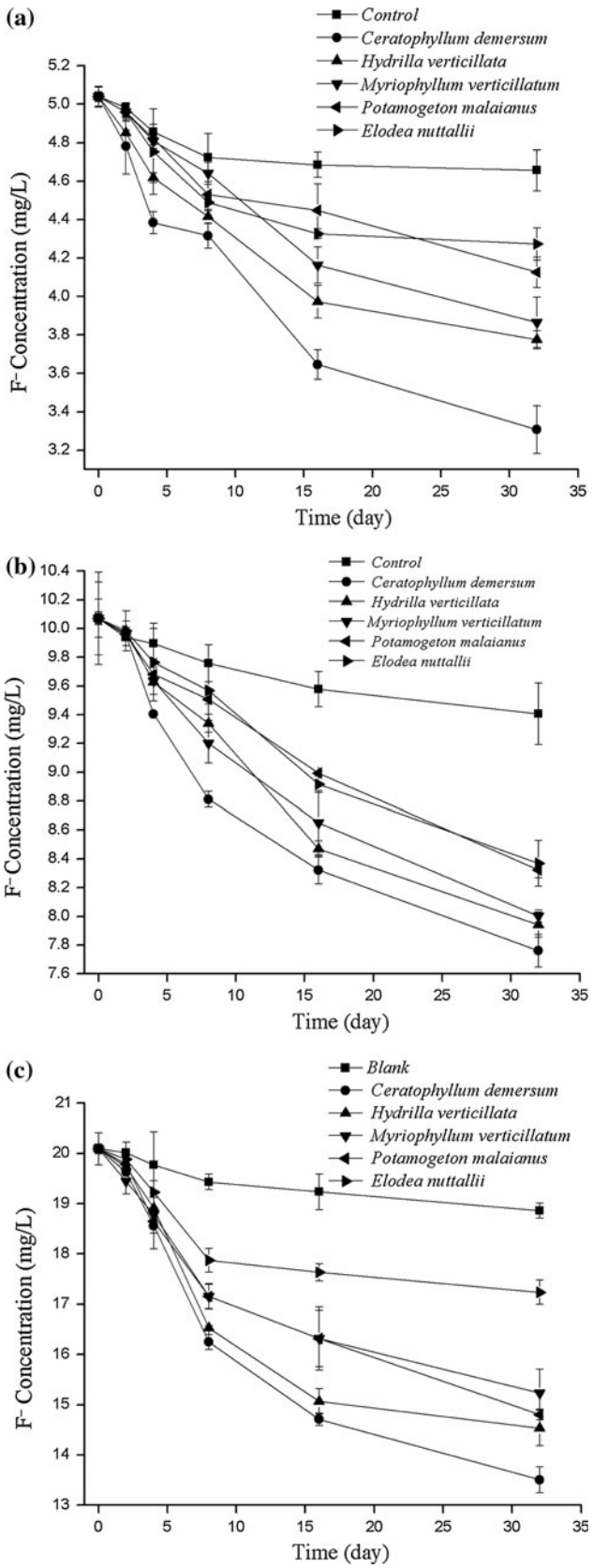
For plant dry weight determinations, five aquatic plant samples were first rinsed and cleaned with tap water, then with deionized water. Samples were then oven-dried at 105°C for 20 min to deactivate enzymes, followed by drying at 70°C for 48 h to constant weight (dry weight). The relative growth rate (RGR) was calculated by the following formula: $RGR = (Wt_{32} - Wt_0)/Wt_0$ (Wt_0, Wt_{32} were the dry weights at days 0 and 32, respectively).

To estimate plant F^- content, 2.0–5.0 g dried plant samples were first pulverized, and then soaked in crucibles for 30 min with 5 mL 10 % $Mg(NO_3)_2$ and 0.5 mL 10 % NaOH. They were then evaporated to dryness in a water bath, carbonized under low temperature (315°C), and ashed for 6 h in a muffle furnace (600°C). The cooled crucibles were washed several times with deionized water, and the rinses of these samples were combined in 25 mL volumetric flasks. The F^- content in each sample was estimated by ion chromatography. The reproducibility of the instrument was checked and the F^- estimation in plants gave 95 %–97 % recovery.

Table 1 Chemical components and prescription of culture solution

Chemical component		Reagent	
Components	Concentration (mg L^{-1})	Reagent	Concentration (mg L^{-1})
Ca	20.0	$CaCl_2$	55.5
Mg	4.8	$MgSO_4 \cdot 7H_2O$	49.0
K	19.7	A–Z solution	52.0
SO_4	21.1	Ferrum tartrate	0.1(ml L^{-1})
Cl	55.8	KH_2PO_4	13.6
P	3.1	KNO_3	51.0

A–Z solution: H_3BO_3 (0.286 mg L^{-1}), $CuSO_4 \cdot 5H_2O$ (0.008 mg L^{-1}), $ZnSO_4 \cdot 7H_2O$ (0.022 mg L^{-1}), $MnCl_2 \cdot 4H_2O$ (0.0181 mg L^{-1}), $H_2MoO_4 \cdot 4H_2O$ (0.009 mg L^{-1}). Ferrum tartrate: heating Na_2EDTA, join $FeSO_4 \cdot 7H_2O$ solution, stirring constantly, cooling, constant its volume to 1 L



◀ **Fig. 1** F^-concentration (mg L^{-1}) of the water in **a** 5 mg F^-/L, **b** 10 mg F^-/L and **c** 20 mg F^-/L culture solution over the study period. Results are mean ± standard deviation ($n = 3$)

Results and Discussion

F^- toxicity in plants is normally manifested by marginal necrosis (tip-burn, scorching, or lesions) on foliage, which begins on the margin or tips of the leaves and moves inward (Brewer, 1966). The F^- solutions induced different toxic effects in the five species of plants. Observations of chlorosis were noted in *E. nuttalli*, necrosis in *P. malaianus*, and moderate decay in *M. verticillatum*. No readily visible signs of toxic effects were apparent in *C. demersum* or *H. verticillata*. However, all five species were able to remove F^- from water at concentrations of 5–20 mg L^{-1}.

As shown in Fig. 1, the five submerged plants can be effectively used in reducing F^- concentration of water. At 5 mg F^-/L, except for *E. nuttallii* and *P. malaianus*, F^- concentration in other three species declined significantly after being cultured for 32 days, and tended to level off after 16 days. Relative to the controls, *C. demersum* exhibited the greatest removal of F^- in the experiment at 27.0 %, compared to 17.6 %, 15.8 %, 10.6 % and 7.7 % of *H. verticillata*, *M. verticillatum*, *P. malaianus* and *E. nuttallii*, respectively (Fig. 1a). The reductions of F^- observed in the control samples indicated the possibility of biochemical and physico-chemical processes functioning in the system. F^- uptake did not show any apparent saturation at 32 days, indicating that higher F^--removal could be possible at longer exposure time. At 10 mg F^-/L experiment (Fig. 1b), the F^--removal effect of all tested species ranked as *C. demersum* (16.5 %), *H. verticillata* (14.7 %), *M. verticillatum* (14.1 %), *P. malaianus* (10.8 %) and *E. nuttallii* (7.4 %). In the presence of 20 mg F^-/L, F^--removal of *C. demersum* reached 26.8 %, compared to 21.6 %, 18.2 %, 20.3 % and 8.1 % F^--removal by *H. verticillata*, *M. verticillatum*, *P. malaianus* and *E. nuttallii*, respectively. The maximum F^--removal by *H. verticillata* was 24.4 % at 2.5 mg F^-/L (Sinha et al. 2006), while F^--removal by *C. demersum* could reach 27.0 % at 5 mg F^-/L, which showed that *C. demersum* had better removal ability than *H. verticillata*, the result was in accordance with our research. For *C. demersum*, F^- uptake did not show any apparent saturation at 32 days (Fig. 1c), indicating that higher F^--removal could be possible at longer exposure time.

Among the five species selected for the study of the kinetics of F^- removal, under the three F^- concentrations, *C. demersum* consistently exhibited the highest F^- removal rate from the water. A comparison of the slopes in Fig. 1 also indicates that the removal of F^- appeared to have

Table 2 Content of F^- in *C. demersum, H. verticillata, M. verticillatum, P. malaianus and E. nuttallii* before and after the experiment (μg g^{-1} dry weight)

Sampling time	F^- Conc. (mg L^{-1})	F^- Content in submerged plants corpus (μg g^{-1} dw)				
		C. demersum	*H. verticillata*	*M. ericillatum*	*P. malaianus*	*E. nuttallii*
Before experiment	_	33 ± 3.1	138 ± 20.4	26 ± 4.3	33.9 ± 3.7	25.8 ± 23.0
After experiment	0	26 ± 2.2	129 ± 15.4	23.62 ± 3.7	30.6 ± 56.9	22.6 ± 14.0
	5	989 ± 21.3	756 ± 5.3	942 ± 6.4	818 ± 36.7	942 ± 16.0
	10	1595 ± 32.6	1563 ± 20.4	1435 ± 6.9	1406 ± 25.1	1030 ± 2.0
	20	1889 ± 26.3	1936 ± 14.4	1437 ± 15.3	1557 ± 11.2	1398 ± 3.0

Results are mean ± standard deviation ($n = 3$)

slowed somewhat for the other species, particularly at F^- concentrations of 5 and 20 mg/L. F^- uptake by *E. nuttalli* was the slowest. However, all species continued accumulating F^- until day 32 without apparent saturation.

Considerable F^- accumulation in plant samples collected from an oxbow lake reservoir along with a lack of distinct disease symptoms, may indicate high tolerance of *M. verticillatum* and *C. demersum* to bioaccumulation of F^- (Pinskwar et al. 2006). Sequestration of the anion in cell vacuoles may be one possible mechanism of F^--tolerance.

As shown in Table 2, the five submerged plant species cultivated in the 0 mg F^-/L water for 32 days had a minus value of F^--accumulation, perhaps because of plant growth dilution. The hornwort *C. demersum* L. cultivated in the 5, 10, 20 mg F^-/L culture solutions for 32 days exhibited the best F^--accumulation ability. A comparison of the values in Table 2 also indicates that the other species appeared to accumulated lower amounts of F^- somewhat, especially the waterweed *E. nuttallii* L. Besides, species appeared to accumulated lower amounts of F^- in lower F^--concentration culture. Similar findings are reported by Ruan, who summarized that F^--uptake by *Camelia sinensis* L. tea plants was linearly correlated to external F^--concentration (Ruan et al. 2003).

Considerable F^- accumulation in plant samples cultivated in the 5, 10, 20 mg F^-/L water for 32 days along with a lack of distinct disease symptoms indicate high tolerance of F^- by *C. demersum* and *H. verticillata*, while the lowest amounts of F^- accumulation by *E. nuttallii* indicate its poor tolerance. However, this is only a hypothesis that needs to be verified.

The growth of five species of submerged macrophytes was favorable during the study periods (Fig. 2). At 5 mg F^-/L culture solution, *C. demersum*, *H. verticillata, M. verticillatum* and *P. malaianus* canadensis grew rapidly, which is reflected by their respective RGR values of 0.28, 0.19, 0.15 and 0.15. Since the optimal growing seasons for *E. nuttallii* were July and August, the plant propagules were found and the plant decayed normally at the end of the experiments, which caused minus growth of biomass in the later period of the experiment. Therefore, the RGR of *E. nuttallii* was the lowest (0.035) among the five submerged plants. At 10 mg F^-/L culture solution, submerged plant species had lower RGR values than at 5 mg F^-/L. However, *C. demersum* still had the highest RGR (0.14), followed closely by *H. verticillata* (0.12). *M. verticillatum* and *P. malaianus* had lower RGR values of 0.06 and 0.07, respectively. *E. nuttallii* had its leaves withered in the later portion of the study due to the season of the year, so its RGR was the lowest (−0.006) among the five species. At the highest F^- concentration of 20 mg/L, the RGR declined significantly (Fig. 2, $p < 0.01$), indicating an obvious toxic effect on growth. The RGR values for *C. demersum*, *H. verticillata*, *M. verticillatum* and *P. malaianus* were 0.10, 0.06, 0.04 and 0.02, respectively. Because of the same reason mentioned above, the *E. nuttallii* plants decayed normally at the end of the experiment, which caused minus growth of biomass in later portion of the experiment. Therefore, the RGR of *E. nuttallii* was the

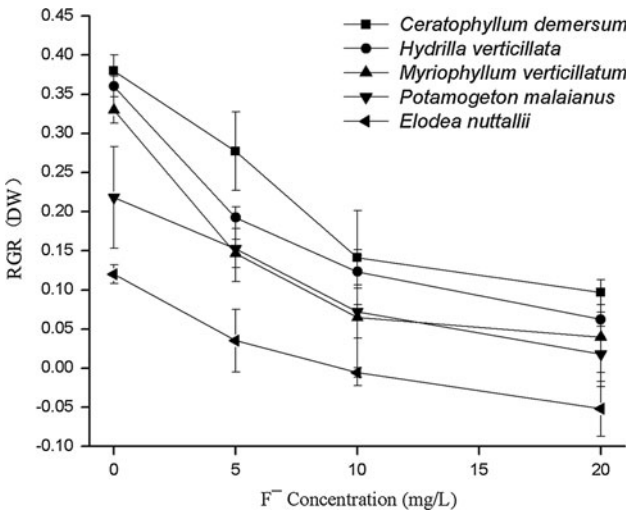


Fig. 2 RGR of five submerged plants in different F^- concentrations. Results are mean ± standard deviation (n = 3). $F_{SPECIES} = 96.55 (p < 0.01)$, $F_{CONCENTRATIONS} = 247.80$ ($p < 0.01$) and $F_{SPECIES*CONCENTRATIONS} = 4.24$ ($p < 0.01$) according to two-way ANOVA. SPECIES = the spicies treatment, CONCENTRATIONS = the concentrations treatment

lowest (−0.05). The toxic effect to *C. demersum* was not so obvious and fast as the other four submerged plants. Its RGR decreased by 26.3 %, 63.2 %and 73.7 % from controls at 5, 10 and 20 mg F/L, respectively. Therefore, it may be necessary to use concentrations greater than 20 mg/L when evaluating the toxic effects of F^- on *C. demersum*.

In this study, we confirmed a relationship among F^- concentrations in water, aquatic plants' dry weight RGR and F^- bioaccumulation in the aquatic plants. At a given exposure time, increasing F^- concentrations in the exposure water resulted in increased bioaccumulation of F^- in plant tissue, and decreased RGR values. The observed results suggested that even greater removal of F^- may have been possible with longer exposure times.

Ceratohyllum demersum appears to possess a high level of tolerance to F^- toxicity based upon its F^--removal performance, F^- bioaccumulation, and RGR at different F^- concentrations in the water. Therefore, it may be a desirable species for cultivation in water with high F^- concentrations, For example, it could be applied in constructed wetlands in high fluoride area. Further studies will be required to determine the specific F^--tolerance mechanism(s) of *C. demersum*.

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