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Fluoride Removal from Water by *Hydrilla verticillata* (l.f.) Royle and Its Toxic Effects

S. Sinha, R. Saxena, S. Singh

Environmental Sciences Division, National Botanical Research Institute, Lucknow, India

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Fluoride, the ionic form of fluorine is widely distributed in nature. It is a common constituent of most soils and rocks. The industrial effluent and sewage discharged from the domestic water supplies supplemented with fluoride contribute to the fluoride levels in aquatic systems. Combustion of coals and volcanic activity also contribute fluorine containing dusts and gases to the atmosphere. During rainfall, these get dissolved in water and contaminate the water bodies. Fluoride has been considered both as an essential element and potent environmental pollutant at high concentrations causing a number of disorders (fluorosis) among the consumers. Fluorosis in general, has been identified in various countries. In India, the problem of high fluoride content (0.5-8.0 $\mu g\ ml^{-1}$) in drinking water resources and consequently a higher incidence of fluorosis (Chand, 1998, Susheela *et al.* 1992,1993) due to high intake of water is very much common. In India, most of the water bodies are highly contaminated by fluoride with varying concentrations in the range of 0.5-20mg L^{-1} (Rajagopal and Tobin, 1991, Sarma and Rao, 1997). Within last few years, the plant based phytoremediation approach to improve the quality of water has become an area of intense study. Phytoremediation is recognized as a cost-effective and environmental friendly option for clean up of contaminated water. In addition, it may make possible to treat the contaminants *in situ*. Not much attention has been paid for the removal of fluoride from water bodies using aquatic macrophytes. The present study has been carried out to remove fluoride using a submerged plant, *Hydrilla verticillata* (l.f.) Royle under laboratory and field conditions. The effect of fluoride on chlorophyll, protein, cysteine and malondialdehyde contents has been studied under laboratory conditions.

MATERIALS AND METHODS

The plants of *Hydrilla verticillata* (l. f.) Royle were collected from unpolluted water bodies in and around Lucknow and acclimatized for 6 months in laboratory campus. Healthy plants were cut off from acclimatized mother plants and care was taken to use the plants with almost the same biomass (3.0g fresh weight). These plants were acclimatized in 10% Hoagland's solution for 6 weeks under laboratory conditions. The different concentrations of fluoride (1.0, 2.5, 5.0, 7.5, 10, 15, 20 and 25 ppm) were prepared in 10% Hoagland's solution using sodium

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Correspondence to: S. Sinha

fluoride. Three sets of each concentrations (three separate beakers for each concentrations served as three replicates) were kept in 250 ml beaker (200 ml solution) containing different concentrations (1, 2.5, 5.0, 7.5, 10 ppm) of fluoride in 10% Hoagland's solution. The fluoride concentrations were measured in each beaker. The plants with almost the same biomass (3.0 g fresh weight, two plant fragments) were kept in all the treatment and control sets under submerged conditions. Plants in 10% Hoagland's solution served as the control. The experiment was performed under standard physiological conditions providing 14 h per day fluorescent light of 114 μ moles m^{-2} s^{-1} intensity at 26 ± 2°C temperature. One set of the plant was harvested after 3, and remaining two sets after 5 and 7 days, respectively. The solution initial pH 6.4, 6.4, 6.42, 6.42, 6.44, 6.66 changed to 7.90, 8.39, 8.39, 8.42, 8.42, 8.98 at 1.0, 2.5, 5.0,7.5, 10, 15 ppm of fluoride, respectively after 3 d without further any change in pH. Simultaneously, an another set of experiment was carried out using high concentrations (15, 20, 25 ppm) of fluoride. These plants were harvested after 7 days. For the removal of fluoride under field conditions, the plants of *H. verticillata* (3.0 gm) were kept in 250 ml beaker containing 200 ml water, collected from four different water bodies of Lucknow. Similarly, another experiment was carried out without plants to assess chemical control of fluoride. Its estimation in each concentration showed no loss of fluoride due to sorption to the walls of the beaker or loss to the atmosphere.

In laboratory experiments, part of the harvested plants were blotted using blotting paper to remove excess water and used for the estimation of chlorophyll, protein, cysteine and malondialdehyde contents. For the estimation of fluoride content in plants, remaining harvested plants were washed thoroughly with distilled water, dried in an oven at 70° C, digested with 70% HNO_3 in Microwave Digestion system (MDS 2000). The digested samples were neutralized with 6N NaOH for the estimation of fluoride. The solution and water samples (50 ml) were mixed with 5 ml of TISAB III (total ionic strength adjustment buffer) supplied along with the instrument to dissociate F-complexes and stabilize pH. Fluoride was estimated using Orion Expandable Ion analyzer model EA^{TM} 940 with a fluoride ion selective electrode. Sodium fluoride was used for the preparation of standard solutions. The reproducibility of the instrument was checked. The fluoride estimation in plants gave 95-97% recovery.

The effect of fluoride in the plants of *H. verticillata* was studied on chlorophyll, protein, cysteine and malondialdehyde contents. Chlorophyll of control and treated plants was extracted in 80% chilled acetone and estimated using a Uvikon 930 spectrophotometer following the method of Arnon (1949). Protein was measured by the method of Lowry *et al.* (1951) using BSA as standard. Cysteine content was measured in fresh plants (500 mg) by the method of Gaitonde (1967). The level of lipid peroxidation was measured in terms of malondialdehyde (MDA), a product of lipid peroxidation and estimated by the thiobarbituric acid reaction (Heath and Packer 1968). The concentration of MDA was calculated using the extinction coefficient of 155 mM^{-1} cm^{-1} and by correcting for the specific absorbance at 600 nm (532-600 nm). Student's 't' test was applied to see

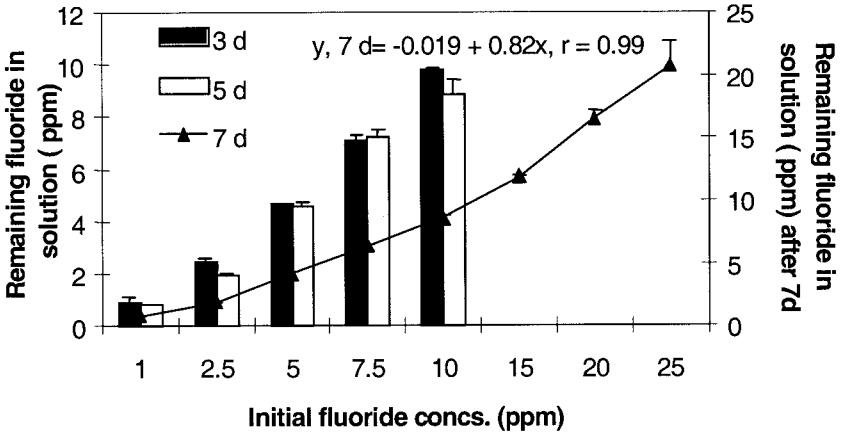

Figure 1. Fluoride concentrations (ppm) in remaining water after harvesting the plants of *H. verticillata* under laboratory conditions. Values are means n=3 ± S.D.

the statistical significance of the results as compared to the control (Gomez and Gomez, 1984).

RESULTS AND DISSCUSSION

The removal of fluoride by the plants of *H. verticillata* is shown in Fig 1. The removal efficiency increased with increase in exposure period. The maximum removal 24.4% was found at 2.5 ppm of fluoride after 7 d. The accumulation of fluoride increased with increase in fluoride concentrations (Table 3) showing maximum accumulation of 1892 $\mu g\ g^{-1}$ dw at 20 ppm after 7 d. At prolonged exposure up to 10 d, the plants showed leaching of fluoride in water at 25 ppm which is evident from 8.8% removal of fluoride after 10 d instead of 17.28% removal after 7 d. At higher concentration (40 ppm), the plant showed visible symptom of toxicity and 13.8% removal was found after 10 d (data not shown in Table). The water samples were collected from four sites of Lucknow, and have shown high fluoride content (Table 4). The results showed the removal of fluoride from the water by the plants of *H. verticillata* which increased with increase in duration up to 7 d followed by leaching.

The effect of fluoride on chlorophyll contents is shown in Table 1. The chlorophyll content increased with increase in concentration of fluoride up to 3 d of exposure at all the concentrations. A maximum increases of 55.9% ($p < 0.05$), 54.7 ($p < 0.05$), 44.2 ($p < 0.1$) and 83.5% ($p < 0.025$) were found in total chlorophyll, chlorophyll- a, chlorophyll- b and carotenoid contents, respectively at 10 ppm after 3 d of exposure period as compared to control. The maximum decreases of 27.8 ($p < 0.1$), 27.6 ($p < 0.1$), 28.3 ($p < 0.1$) and 24.5% ($p < 0.1$) were found in total chlorophyll, chlorophyll- a, chlorophyll- b and carotenoid contents, respectively at 25 ppm of fluoride after 7 d of exposure as compared to control (Table 2). The effect of different fluoride concentrations on

Table 1. Effect of fluoride on total chlorophyll, chlorophyll-a, chlorophyll-b, and carotenoid contents (mg g^{-1} fw) in *H. verticillata.*

Conc. (ppm)	Exposure (days)			Chlorophyll Content (mg g^{-1} fw)
	3	5	7	
0.0	1.62 ± 0.34	1.65 ± 0.19	1.64 ± 0.20	Total Chl
	1.11 ± 0.22	1.13± 0.16	1.12 ± 0.10	Chl- a
	0.53 ± 0.12	0.56 ± 0.07	0.55 ± 0.06	Chl-b
	0.44 ± 0.32	0.44± 0.03	0.44 ± 0.12	Carotenoid
1.0	1.75 ± 0.14	1.59 ± 0.66	1.57 ± 0.12	Total Chl
	1.18 ± 0.10	1.01 ± 0.48	1.05 ± 0.11	Chl- a
	0.58 ± 0.05	0.52 ± 0.25	0.50 ± 0.04	Chl-b
	0.63 ± 0.08	0.42 ± 0.09	0.43 ± 0.02	Carotenoid
2.5	1.73 ± 0.74	1.81 ± 0.21	1.49 ± 0.09	Total Chl
	1.50 ± 0.33	1.22 ± 0.19	1.05 ± 0.11	Chl- a
	0.74 ± 0.12	0.60 ± 0.10	0.47 ± 0.02	Chl-b
	0.70 ± 0.22	0.46 ± 0.08	0.41 ± 0.02	Carotenoid
5.0	1.76 ± 0.14	1.88 ± 0.08	1.46 ± 0.09	Total Chl
	1.49 ± 0.11	1.27 ± 0.10	1.00 ± 0.06	Chl- a
	0.57 ± 0.05	0.61 ± 0.04	0.47 ± 0.04	Chl-b
	0.61 ± 0.04	0.48 ± 0.05	0.41 ± 0.02	Carotenoid
7.5	2.36 ± 0.52	1.78 ± 0.80	1.40 ± 0.27	Total Chl
	1.60 ± 0.35	1.20 ± 0.16	0.99 ± 0.81	Chl- a
	0.77 ± 0.17	0.58 ± 0.07	0.47 ± 0.09	Chl-b
	0.78 ± 0.08	0.47 ± 0.07	0.38 ± 0.02	Carotenoid
10.0	2.55 ± 0.18[a]	1.66 ± 0.31	1.30 ± 0.04[a]	Total Chl
	1.72 ± 0.10	1.18 ± 0.17	0.99 ± 0.04[a]	Chl- a
	0.76 ± 0.08	0.60 ± 0.13	0.47 ± 0.03[b]	Chl-b
	0.80 ± 0.04	0.47 ± 0.04	0.39 ± 0.004[c]	Carotenoid

Values are mean of triplicate ± S.D. t-test (one tailed as compared to control) [a] = $p<0.05$; [b] = $p<0.1$; [c] = $p<0.025$. (Total Chl.) y, 3 days = 1.56+0.093x, r = 0.93; (Total Chl.) y, 5 days = 1.69+0.0068x, r = 0.24; (Total Chl.) y, 7 days = 1.60-0.030x, r = -0.98

protein content at different exposure period is shown in Fig 2 and at higher concentrations of fluoride (Table 2). It showed decrease in protein content with an increase in fluoride concentrations and treatment durations. A maximum decrease of 40.8% was found at 25 ppm of fluoride after 7 d of exposure as compared to control. The effect of fluoride on cysteine content after 7 d is shown in Table 3. The cysteine content increased significantly with increase in concentration. The level of lipid peroxidation was measured in terms of malondialdehyde (MDA), a

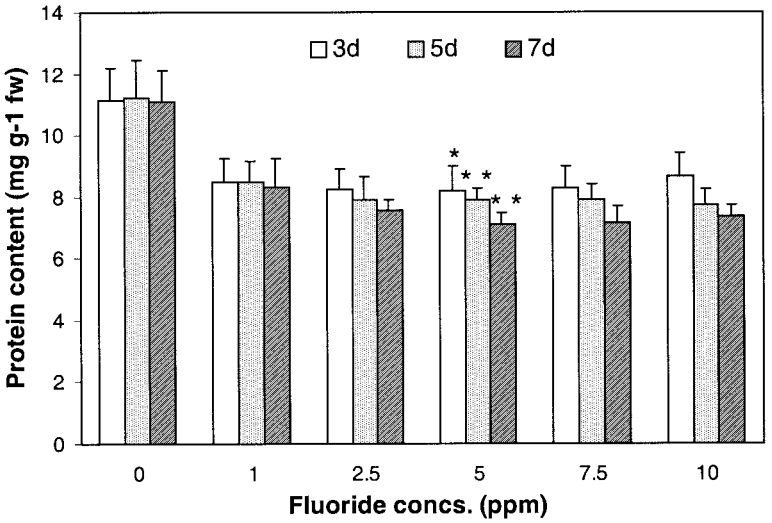

Figure-2. Effect of Fluoride on protein content (mg g^{-1} fw) in *H. verticillata* at different concentrations. All the values are mean of three replicates ± S.D. t-test (one tailed as compared to control) *= p<0.025; **=p<0.01.

Table 2. Effect of fluoride on total chlorophyll, chlorophyll a, chlorophyll b, carotenoid and protein contents (mg g^{-1} fw) in *H. verticillata* after 7 days.

Conc. (ppm)	Total chl	Chl a	Chl b	Carotenoid	Protein
0.0	1.63± 0.48	1.12± 0.33	0.54± 0.15	0.44± 0.14	10.98±0.98
15	1.30± 0.53	0.96± 0.37	0.46± 0.05	0.37± 0.16	7.10±0.62
20	1.28± 0.20	0.85± 0.14	0.46 ± 0.05	0.35± 0.03	7.00±0.39[b]
25	1.18± 0.33[a]	0.81±0.32[a]	0.39± 0.09[a]	0.33± 0.01[a]	6.50±0.49[c]

Values are mean of triplicate ± S.D. t-test (one tailed as compared to control) [a], p<0.05; [b], p<0.025; [c], p<0.01. (Total Chl.) y, 7 days = 1.62-0.018x, r = -0.99

product of lipid peroxidation in the plant samples. A decrease in MDA content (Table 3) was found in the fluoride treated plants.

Cooke *et al.*, (1976) reported that the flora naturally established at deposits of fluorspar mine waste contain high concentrations of fluoride ranging between 100 and 10,000 μg F/g dw. Boese *et al.*, (1995) reported that treated leaves of spinach with 2 mM NaF for 3 and 5 hours resulted in the accumulation of 243 and 412 ppm F, respectively. Shirke and Chandra (1991) reported that the plants of *Spirodela polyrrhiza* grows luxuriantly in ponds around Lucknow, was found to contain high levels of fluoride in fields and laboratory conditions. The maximum uptake of fluoride was 918.6 μg g-1 dw at 20 ppm after 7 days by the fronds of *S. polyrrhiza*. In the present study, the accumulation of fluoride was found more

Table 3. Mean concentration of fluoride (μg g^{-1} dw) in *H. verticillata* and its effect on cysteine (n mol g^{-1} fw) and MDA (n mol g^{-1} fw) contents after 7 days.

Fluoride Conc. (ppm)	Accumulation (μg g^{-1} dw)	Cysteine (n mol g^{-1} fw)	MDA content (n mol g^{-1} fw)
0.0	-	85.360 ± 2.6	11.30 ± 1.005
1.0	153 ± 6.9	84.320 ± 1.9	8.98 ± 0.365
5.0	987 ± 20.6	95.440 ± 2.2	9.30 ± 0.705
10.0	1067 ± 18.6	113.59 ± 2.6	8.92 ± 1.17
20.0	1892 ± 25.3[a]	118.52 ± 3.9[b]	7.82 ± 0.44[c]

Values are mean of triplicate ± S.D. t-test (one tailed as compared to 1 ppm) [a], $p<0.0005$. Student t-test (one tailed as compared to control) [b], $p<0.005$; [c], $p<0.025$. (Accumulation) y, 7 days = 280.38+82.71x, r = 0.95; (Cysteine) y, 7 days = 86.28+1.83x, r = 0.94; (MDA) y, 7 days = 10.14-0.12x, r = -0.78

Table 4. Fluoride concentration (ppm) and pH in the remaining water (collected from different water bodies of Lucknow) after harvesting the plant of *H. verticillata* at different duration.

Site	Fluoride Concentration				
	0 d	3 d	5 d	7 d	15 d
Basti	3.59 ± 0.07 (7.32)	3.31 ± 0.01 (8.50)	2.91 ± 0.02 (8.63)	2.78 ± 0.01 (8.51)	3.19 ± 0.01 (8.68)
Anora	4.72 ± 0.06 (7.92)	4.59 ± 0.02 (8.59)	4.15 ± 0.02 (8.97)	3.88 ± 0.03 (8.48)	4.20 ± 0.01 (8.60)
Jafar nagar	8.61 ± 0.11 (7.59)	8.43 ± 0.07 (8.48)	7.53 ± 0.02 (8.98)	7.43 ± 0.05 (8.77)	8.11 ± 0.01 (8.60)
Papna-mau	2.26 ± 0.07 (7.68)	2.12 ± 0.01 (8.17)	1.82 ± 0.01 (8.52)	1.70 ± 0.03 (8.42)	2.09 ± 0.01 (8.59)

Values are mean of triplicate ± S.D. pH values are in parenthesis.

(1892 μg g-1 dw) at the same concentration and duration and increased with increase in fluoride concentration. This may be due to more surface area of the plants of *H. verticillata* (submerged) than *S. polyrrhiza* (free floating). Similar findings have been reported in other rooted submerged plants in relation with the metal accumulating potential (Gupta *et al.*, 1994). Pendias and Pendias (1984) also found that accumulation of fluoride in plants increases with increasing fluoride concentrations.

Shirke and Chandra (1991) reported that there was no effect on chlorophyll and protein contents in *Spirodela polyrrhiza* (free floating) after treatment with fluoride (up to 25 ppm) as compared to control. Boese *et al* (1995) also reported that fluoride treatment (2 mM) had no effect on the chlorophyll content in the leaves of spinach after 24 hours as compared to control. In contrast, the chlorophyll and protein contents decreased with increase in fluoride

concentrations in the plants of *H. verticillata* (submerged). The use of two different types of aquatic plant account for the difference in toxicity.

Fluoride is known to increase the level of free amino acids. Hautala and Holopainen (1995) reported that fluoride treatment (100 and 200 mg/l) significantly increased the level of individual free amino acids in barley foliage. The results of the present study are in agreement with the findings of these authors showing increase in cysteine content with increase in fluoride concentrations. This may be due to synthesis of fluoride induced protein or enzymes as reported by Yang and Miller (1963). Shayiq et al., (1986) reported significant inhibition of lipid peroxide in liver and intestine of rat after treatment with 10 mM NaF. The reason for inhibition of lipid peroxidation product formation by fluoride could also be due to stimulation of reducing capacity of plant tissue by increasing cysteine content which protect the membrane from oxidative attack of oxygen free radicals under natural conditions (Bus et al., 1976). This may be the reason showing decrease in malondialdehyde content in the present study.

The results indicate that the plants of *H. verticillata* are found effective in removing fluoride from contaminated water under laboratory and field conditions. The high fluoride concentration affected the chlorophyll and protein contents. However, there was no visible injury seen in the plants up to 25 ppm of fluoride.

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REFERENCES

Arnon DI (1949). Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta valgaris*. Plant Physiol 24:1-15

Boese SR, MacLean DC, EI-Mogazi D (1995). Effect of fluoride on cholorophyll-a fluorescene in spinach. Environ Pollut 89:203-208

Bus JS, Aust SD, Gibson JE (1976). Paraquat toxicity : proposed mechanism of action involving lipid peroxidation. Environ Hlth Prospect 16:139-146

Chand D (1998). Fluoride and human health-A cause for concern. Indian J Environ Pollut 19: 81-89

Cooke JA, Johnson MS, Davison AW, Bradshaw AD (1976). Fluoride in plants colonising fluorspar mine waste in the Peak District and weardale. Environ Pollut 11:10-23

Gaitonde MK (1967). A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids. Biochem J 104:627-633

Gomez KA, Gomez AA (1984). Statistical procedures for agricultural research. John. Wiley & Sons, New York

Gupta M, Sinha S, Chandra P (1994). Uptake and toxicity of metals in *Scirpus lacustris* L.and *Bacopa monnieri* L. J Environ Sci Health 29(10): 2185-2202

Hautala EL, Holopainen JK (1995). Gramine and free amino acids as indicators of fluoride- induced stress in barley and its consequences to insect herbivory. Ecotoxicol Environ Saf 31: 238- 45

Heath RL, Packer L (1968). Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 196:385-395

Kabata-Pendias A, Pendias H (1984).Trace elements in soils and plants Chapter 12. CRC Press pp 384

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin-phenol reagent. J Biol Chem 193:265-75

Rajagopal R, Tabin G (1991) Fluoride in drinking water. A survey of expert opinions. Environ Geochem Health 13:3-13

Sarma DRR, Rao SLN (1997) Fluoride concentrations in ground waters of Visakhapatnam, India Bull Environ Contam Toxicol 58:241-247

Shirke PA, Chandra P (1991) Fluoride uptake by Duckweed *Spirodela polyrrhiza.* Fluoride 24(3):109-112

Shayiq RM, Raza H, Kidwai AM (1986) Fluoride and lipid peroxidation : A comparative study in different rat tissues. Bull Environ Contam Toxicol 37:70-76

Susheela AK, Das TK, Gupta IP, Tandon RK, Kacher SK, Goshi RC, Deka RC, (1992) Fluoride ingestion and its correlation with Gastro-intestinal discomfort. Fluoride 25:5-21

Susheela A, Kumar A, Bhatnagar M, Bahadur, Rashimi (1993) Prevalence of endemic fluorosis with gastro-intestinal manifestation in people living in some North Indian village. Fluoride 26:94 - 104

Yang SF, Miller GW (1963) Biochemical studies on the effect of fluoride on higher plants 1. Metabolism of carbohydrates, organic acids and amino acids. Biochem J 88:505-515