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Phytoremediation of fluoride contaminated water and soil: A search for fluoride hyperaccumulators

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Fluoride (F) is a common environmental pollutant and F rich soil is a potential source of its contamination in groundwater, into the food chain and finally also into human body. Screening for F hyperaccumulators can be of great help in phytoremediation of F. The present study was undertaken to investigate the potential of eight tree species of semi arid region *viz*. *Acacia tortilis, Acacia nilotica, Acacia senegal, Prosopis cineraria, Prosopis juliflora, Cassia fistula, Azadirachta indica* and *Albizzia lebbeck* for hyperaccumulation of F. The plants were grown in various concentrations of F *viz*. 5, 10, 15, 20 and 50 mg L^{-1} using hydroponic cultures. Based on the accumulation pattern, three plants *viz*. *A. tortilis, P. juliflora* and *C. fistula* were selected for F uptake and deposition in different organs and their subcellular fractions. Organwise F accumulation studies revealed that roots accumulated maximum F. In general, cytosolic fraction accumulated more F in comparison to cell wall. Among all plant studied, *P. juliflora* accumulated maximum F, whereas *A. senegal* the minimum. The highest F accumulation 2222.83 µg g^{-1} was found in 50 mg L^{-1} F treated 10 days old roots of hydroponically grown *P. juliflora* plants. Our results suggest potential use of *P. juliflora* in excess F removal in soil and water bodies.

Keywords: Fluoride; hyperaccumulator; phytoremediation; hydroponic; *Prosopis juliflora*

Introduction

Fluorides possess considerable potential for causing ecological damage as they are not biodegradable and accumulate in the environment (Marier and Rose, 1977). Moreover, since they are poorly detoxified by plants (Abdallah *et al.*, 2006; Gupta *et al.*, 2009) and animals, they have negative effects on plants, animals and human health through the food chain (Stevens *et al.*, 2000). F is known to cause dental and skeletal fluorosis when its concentration is higher

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than 1.5 mg L^{-1} in drinking water (Teotia *et al.*, 1981; Susheela and Ghosh, 1990).

In view of the widespread distribution of F in high concentrations in drinking water and cultivable soils, it is obvious that immediate measures need to be taken for F removal. The various methods of F mitigation; Nalgonda technique (using alum, lime and bleaching powder to precipitate F salts from water), ion exchange, reverse osmosis, electrolysis (Heidweiller, 1990) etc. are useful for removing F from water but these methods are not suitable for removing F from soil. Besides these conventional techniques, the other potential method is phytoremediation. Phytoremediation is an ecotechnological remediation for treating contaminated soil and water. This phytotechnology uses plants to degrade, transform, assimilate, metabolize, or detoxify hazardous pollutants from soil, aquatic and atmospheric environments. Interest in phytoremediation has grown significantly following the identification of metal hyperaccumulator plant species (Ghosh and Singh, 2005; Shao *et al.*, 2010).

Many indigenous plants are known to absorb excess contaminants from soil/ aquifer and are capable of accumulating exceptionally high concentrations of several elements in their aboveground tissues (Reeves and Baker 2000, Clemens *et al*., 2002; Axtell *et al*., 2003; Rai, 2009) and thus are useful for phytoremediation. Most researches on these so-called hyperaccumulating plants has centered on thorough understanding of mechanisms of uptake, translocation and sequestration (Bligny *et al*., 1972; Ruan *et al*., 2003). Such recent progress has led to valuable insights into the molecular understanding of metal accumulation and tolerance in hyperaccumulating plants (Sinha *et al*., 2000).

Considering no effective plan has been put forward till date about concrete steps of applying a hyperaccumulator to practice, some research groups bring forward novel, tentative and adaptive procedures to evaluate hyperaccumulators feasibility before large-scale commercialization (Mench *et al*., 2010).

The first step towards phytoremediation of F is the search for hyperaccumulators by screening trees and shrubs for tolerance and resistance to F toxicity (Santos and Pendrazaa, 2010). Such species can be raised to remediate F from soil (Kang *et al*., 2008; Wang-Cahill and Fields, 2007). Only such plant species, which accumulate F mainly in roots, are valuable for phytoremediation, as their accumulation in other organs and subsequent consumption by grazing animals or harvest for human may be harmful (Bunce, 1985). Thus it becomes important to determine the F accumulation within different organs of plants *viz*. roots, stem and leaves. Determination of site of accumulation of F within particular organ (Armstrong and Singer, 1980, Yang

et al., 2006) may assist in determining that why this element is toxic in some plants and animals at the levels that are normally present in atmosphere.

The study was an attempt to search for F hyperaccumulators, where F accumulation was studied in eight tree species of semi-arid region of Rajasthan, India.

Experimental

Plant material and their growth

Seeds of eight tree species *viz. A. tortilis, A. nilotica, A. senegal, P. cineraria, P. juliflora, C. fistula, A. indica* and *A. lebbeck* were procured from Central Arid Zone Research Institute (CAZRI), Jodhpur, India.

After surface sterilization, seeds were grown both in Hoagland's nutrient solution (Hoagland and Arnon, 1938) and in soilrite. Soilrite used for experimental study contained a mixture of 75% Sphagnum peat moss and 25% horticulture grade expanded perlite. The pH range was 5-6.5 and the moisture content was 70-75%. For each concentration of F in medium, three independent sets of plants were maintained.

The germinated seeds (10 days old) were grown hydroponically at different NaF concentrations ranging from 0-50 mg L^{-1} (control, 5, 10, 15, 20 and 50 mg L^{-1}). Throughout, the germinating seeds were maintained in a growth chamber at a temperature 30 ± 2^{o} C, light intensity of 1000 lm m^{-2} with 14 h photoperiod and 70% relative humidity.

Surface sterilized and imbibed seeds were sown in plastic trays containing soilrite mixed with a corresponding amount of sodium fluoride (NaF) to give an end concentration of 10, 20, and 50 mg kg^{-1} F. Trays without NaF served as control. Control contained 0.36 µg g^{-1} F in soilrite. The seedlings were grown in green house and maintained at a temperature 30 ± 2^{0}C and 60-65% relative humidity. Six individual seedlings from each species were set up for study.

For F accumulation studies, the seedlings were harvested after 5 and 10 days of F treatment.

Determination of F Content

Hydroponically/soilrite grown seedlings were harvested and F content was determined on whole plant basis. For organwise studies the plants were dissected into different organs i.e., roots, stem and leaves after 5 and 10 days of F treatment. A fraction of F can be absorbed on root surface which is not

considered as root accumulation, therefore, roots were repeatedly washed to remove this fraction of F.

The F content was determined by the method given by Mc-Quaker and Gurney (1977).

Determination of F Content in Cell Wall and Cytosolic Fractions

Cytoplasmic and cell wall fractions were isolated according to the method of Bozarth *et al*. (1987). The comparisons were made between the cell wall and cytosolic fraction of roots, stem and leaves at 5 and 10 days with respect to F accumulation.

The F content in all samples was measured using microprocessor controlled high performance–pH–ion meter, WTW make, model pMX 3000, fitted with a fluoride electrode F 500.

Statistical analysis

All statistical analyses were done using the statistical package of the SAS software computer program. All data were expressed as mean ± standard deviation (S.D.) of three replicates.

Results and discussions

Accumulation of F by different plant species

F accumulation in various plant species grown under different concentrations of F *viz*. 5, 10, 15, 20 and 50 mg L^{-1} for 5 and 10 days is presented in Fig 1. The figure shows that in all plant spp., F content increased with respect to the concentration in the nutrient medium as well as number of days of treatment. Though in all cases maximum accumulation was at 10 days and at 50 mg L^{-1} F concentration, the accumulation pattern in all plant spp. was different.

A. tortilis plants accumulated F rapidly upto 5 days after which accumulation slowed down. Similar pattern was observed in the *P. cineraria* plants kept at 50 mg L^{-1} F and *A. senegal* and *C. fistula* at 20 mg L^{-1} F. In *A. nilotica* the F content increased steadily with an increase in its concentration in the nutrient medium after 5 and 10 days of treatment though the increase at 5 days was not as much as in *A. tortilis*. The accumulation in *A. nilotica* was highest after 10 days of treatment and only after 5 days in *A. tortilis*. The F accumulation was least in *A. senegal. P. juliflora* accumulated maximum F from the nutrient medium (1322 µ g^{-1}dw at 50 mg L^{-1} F after 10 days). *A.*

indica and *A. lebbeck* showed similar pattern of accumulation as *A. nilotica*. In all cases, there was a good correlation (r^2 = 0.90 to 0.99) between total F concentrations in solution and its accumulation by different plants. The results obtained here were similar to the solution culture experiments conducted with F by Bar-Yosef and Rosenberg (1988).

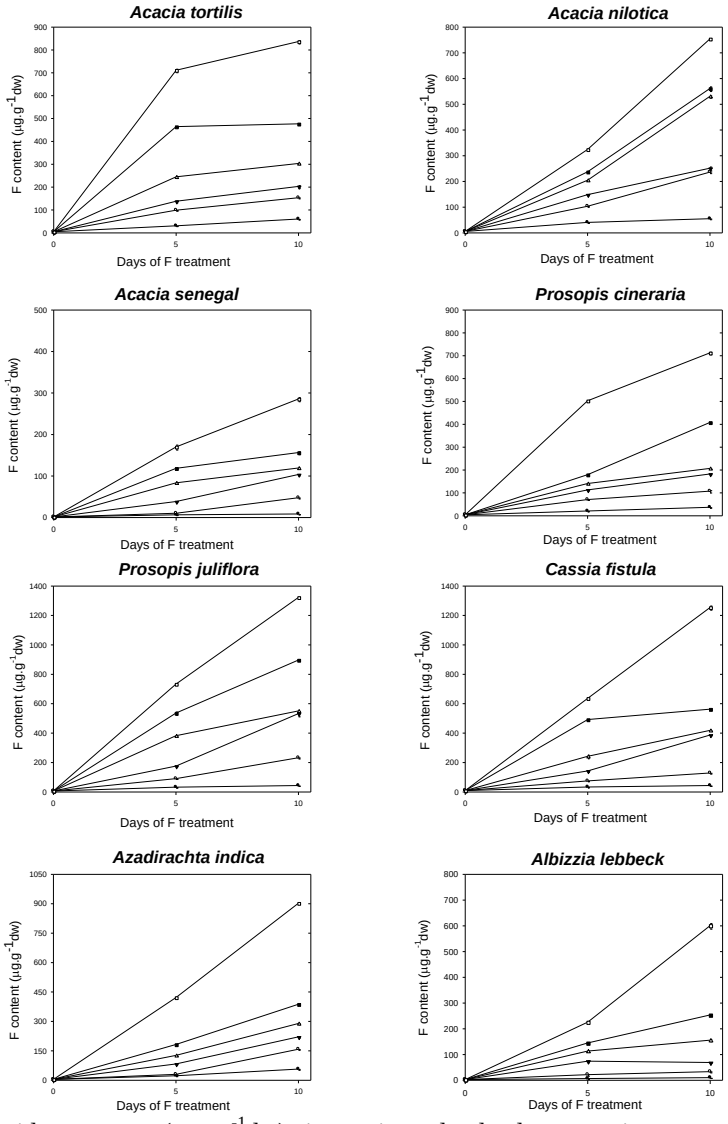

Fig. 1. Fluoride content (µg g^{-1}dw) in various land plant species grown in different concentrations of F, *viz*. control (—●—), 5 µg g^{-1} (—○—), 10 µg g^{-1} (—▼—), 15 µg g^{-1} (—△—), 20 µg g^{-1} (—■—) and 50 µg g^{-1}(—□—) for 0, 5 and 10 days.

The present study revealed an immense variation in the F accumulation capacity within the species e.g. *A. tortilis* had the maximum F accumulating capacity among other *Acacia* species studied. In fact it accumulated *ca.* 4 times more F than *A. senegal*. This intra-specific variation in the uptake of mineral reflected genetically controlled differences in mechanisms of mineral nutrition, especially those concerned with absorption and translocation of a given element (Epstein, 1972). Also, the concentration of a particular element in plant represented the process of uptake, translocation, re-translocation, and utilization. These variations also occurred because of the differences in geographical location, climate, stage of growth and maturation (Vike and Habjorg, 1995).

Organwise F accumulation in three plant species

For organwise distribution studies, only those plant species that accumulated maximum F in them were selected. These were *A. tortilis, C. fistula* and *P. juliflora.*

Roots of *A. tortilis* accumulated maximum F followed by leaves and stem (Table 1). In 5 day old hydroponically grown plants (at 50 mg L^{-1} F), the roots accumulated 8 folds and the leaves 1.6 folds higher F than stem. Similarly in 10 day plants with 50 mg L^{-1} F treatment, F accumulation in roots was *ca.* 3 folds and in leaves it was 1.4 folds higher than stem. For the soilrite grown plants (Table 2), the same pattern of F accumulation was observed i.e. roots > leaves > stem, though the F content in hydroponically grown plants was higher than those grown in soilrite. After 10 days, in soilrite grown plants, the F concentration was almost similar in stem and leaves i.e. 345.13 and 342.47 µg g^{-1}dw.

In *C. fistula* and *P. juliflora*, roots accumulated maximum F and stem the least. At all concentration, a comparison between hydroponically and soilrite grown plants revealed that the increase in F concentration of roots with respect to stem or leaves was highest for hydroponically grown plants. The roots of 10 day F treated plants accumulated about 2223 µg g^{-1} F at 50 µg L^{-1} F treatment (hydroponically grown) which was the highest value obtained.

Within a particular organ, the F accumulation increased with its concentration in the Hydroponic nutrient media or soilrite. For example, in roots of 5 day F treated (50 mg L^{-1}) *A. tortilis* plants, F content increased *ca.* 70 folds in hydroponically grown and 48 folds in soilrite grown plants over the controls.

Table 1. Organwise F accumulation in selected plant species* grown hydroponically in different F concentrations

Plants	Organ	Days after F treatment						
			5 days			10 days		
		F conc.	Moisture	F content[1]		Moisture	F content[1]	
		mg L^{-1}	%	µg g^{-1} dw		%	µg g^{-1} dw	
A. tortilis	Roots	Control	86.07	12.17	± 10.0	83.84	18.33	± 6.4
		10	84.04	278.93	± 11.2	84.06	391.50	± 5.5
		20	83.13	519.20	± 11.9	83.32	683.02	± 11.9
		50	82.10	854.80	± 12.3	83.85	1013.92	± 33.3
	Stem	Control	90.42	13.33	± 5.8	86.42	15.52	± 1.3
		10	88.29	34.40	± 6.9	85.24	91.17	± 18.1
		20	88.62	88.93	± 18.0	84.18	140.17	± 6.3
		50	86.03	105.20	± 4.5	85.84	340.83	± 2.7
	Leaves	Control	85.42	17.33	± 6.8	82.00	12.67	± 2.5
		10	85.28	64.73	± 12.1	82.57	109.17	± 2.0
		20	84.98	81.33	± 8.4	80.67	164.00	± 6.9
		50	84.24	174.27	± 14.4	79.77	491.25	± 25.4
C. fistula	Roots	Control	84.88	16.08	± 4.2	91.01	16.30	± 6.0
		10	84.02	243.30	± 10.2	89.97	333.94	± 13.1
		20	85.40	598.08	± 6.2	88.34	992.06	± 12.5
		50	85.51	845.90	± 17.2	87.39	1480.27	± 22.5
	Stem	Control	90.16	10.12	± 1.9	90.17	12.08	± 2.4
		10	90.25	61.70	± 2.1	89.83	235.56	± 5.0
		20	88.80	120.82	± 2.1	89.63	157.22	± 2.7
		50	89.97	177.93	± 5.8	86.78	316.08	± 2.7
	Leaves	Control	81.76	17.57	± 2.8	87.97	18.92	± 5.5
		10	80.24	122.61	± 8.2	76.12	204.56	± 17.0
		20	78.82	139.86	± 10.8	76.11	191.04	± 4.2
		50	77.20	172.95	± 16.6	80.27	259.92	± 12.9
P. juliflora	Roots	Control	85.56	19.80	± 4.5	81.68	20.34	± 1.8
		10	86.93	291.20	± 14.9	81.54	420.47	± 2.3
		20	86.81	800.93	± 4.0	84.65	1082.27	± 29.5
		50	88.07	1558.27	± 35.9	72.71	2222.83	± 32.0
	Stem	Control	87.66	10.67	± 2.2	87.98	12.24	± 1.1
		10	88.00	196.13	± 6.2	89.56	190.07	± 17.2
		20	89.02	298.13	± 14.7	89.41	336.93	± 41.2
		50	87.35	404.93	± 3.6	88.60	689.33	± 76.2
	Leaves	Control	85.05	19.80	± 2.3	85.49	21.54	± 5.2
		10	84.80	207.73	± 12.2	83.81	241.47	± 12.5
		20	85.69	367.20	± 6.2	86.62	507.13	± 18.5
		50	85.72	728.93	± 3.3	83.31	858.17	± 15.7

* Seedlings were grown hydroponically in Hoagland's nutrient medium.

1 Data are given as mean values ± S.D.

Table 2. Organwise F accumulation in selected plant species* grown in soilrite in different F concentrations

Plants	Organ	Days after F treatment						
			5 days			10 days		
		F conc.	Moisture	F content[1]		Moisture	F content[1]	
		mg kg^{-1}	%	µg g^{-1}dw		%	µg g^{-1}dw	
A. tortilis	Roots	Control	85.33	16.00	± 3.9	83.34	17.73	± 1.8
		10	84.48	248.80	± 3.6	80.21	246.31	± 17.3
		20	82.00	492.40	± 2.1	81.89	497.20	± 12.4
		50	80.72	767.73	± 2.9	82.29	909.80	± 23.6
	Stem	Control	88.53	13.73	± 3.1	82.45	14.80	± 2.1
		10	86.76	29.60	± 3.0	83.23	132.73	± 10.2
		20	85.36	41.60	± 2.8	81.62	215.80	± 33.0
		50	85.35	78.00	± 8.6	85.51	345.13	± 24.4
	Leaves	Control	84.12	12.40	± 3.6	83.81	15.27	± 3.4
		10	84.61	37.20	± 3.7	80.02	169.13	± 6.1
		20	82.23	66.00	± 2.9	79.49	258.27	± 6.5
		50	82.34	123.73	± 14.7	82.81	342.47	± 5.3
C. fistula	Roots	Control	75.30	16.24	± 5.6	88.63	17.03	± 8.0
		10	83.47	180.22	± 5.3	83.75	191.04	± 7.6
		20	75.81	185.70	± 10.8	79.59	269.26	± 5.7
		50	83.39	243.42	± 10.1	79.23	468.17	± 43.8
	Stem	Control	87.60	14.72	± 3.7	89.35	14.33	± 1.2
		10	88.42	85.50	± 6.0	86.18	141.57	± 12.9
		20	87.65	79.01	± 5.6	81.67	158.83	± 10.0
		50	88.29	70.90	± 4.8	82.86	133.00	± 17.5
	Leaves	Control	80.83	15.17	± 2.2	83.25	15.83	± 7.3
		10	80.02	104.16	± 3.9	80.13	116.92	± 8.3
		20	77.78	81.93	± 6.3	75.22	179.08	± 12.5
		50	81.24	64.91	± 6.6	72.71	164.33	± 7.6
P. juliflora	Roots	Control	69.83	15.60	± 1.8	80.41	16.04	± 3.8
		10	62.55	228.00	± 10.4	83.56	335.68	± 10.7
		20	67.47	432.58	± 3.9	84.70	1048.35	± 27.3
		50	71.27	1452.93	± 11.6	84.31	1803.60	± 14.6
	Stem	Control	84.67	10.33	± 0.3	86.81	15.30	± 6.7
		10	82.74	133.60	± 16.5	85.84	171.81	± 4.4
		20	84.36	167.73	± 10.9	87.47	204.78	± 10.7
		50	82.55	191.20	± 7.0	85.48	505.53	± 4.4
	Leaves	Control	81.77	13.87	± 10.5	85.32	17.94	± 1.1
		10	81.21	141.07	± 11.7	83.18	198.81	± 5.1
		20	80.88	113.60	± 3.9	81.72	512.91	± 3.2
		50	81.40	219.33	± 4.7	81.62	896.86	± 17.6

* Seedlings were grown in soilrite.

1 Data are given as mean values ± S.D.

Accumulation in Cell Wall and Cytosolic Fraction

Fig. 2 presents the F content in cell wall and cytosolic fractions of land plants *viz*. *A. tortilis*, *C. fistula* and *P. juliflora*, grown hydroponically for 5 and 10 days. As observed from the figure, there were significant differences in the F content in cell wall and cytosolic fractions of three plants. However, in all these species, the pattern of F accumulation was similar. For example, maximum F was found in cytosolic fraction of roots at 50 mg L^{-1}, followed by leaves and stem.

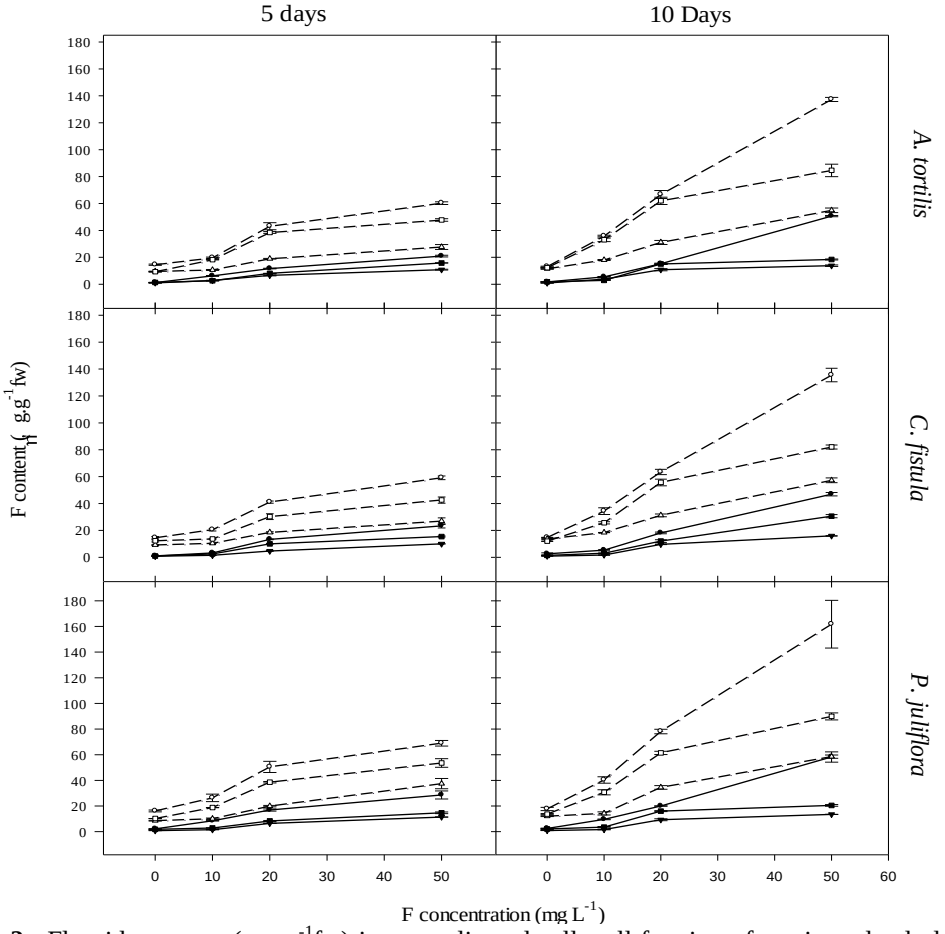

Fig. 2. Fluoride content (µg. g^{-1}fw) in cytosolic and cell wall fraction of various land plants grown hydroponically in different concentrations of F for 5 and 10 days. Root cell wall F ( ), root cytosol F ( ), stem cell wall F ( ), stem cytosol F ( ), leaves cell wall F ( ) and leaves cytosol F ( ).

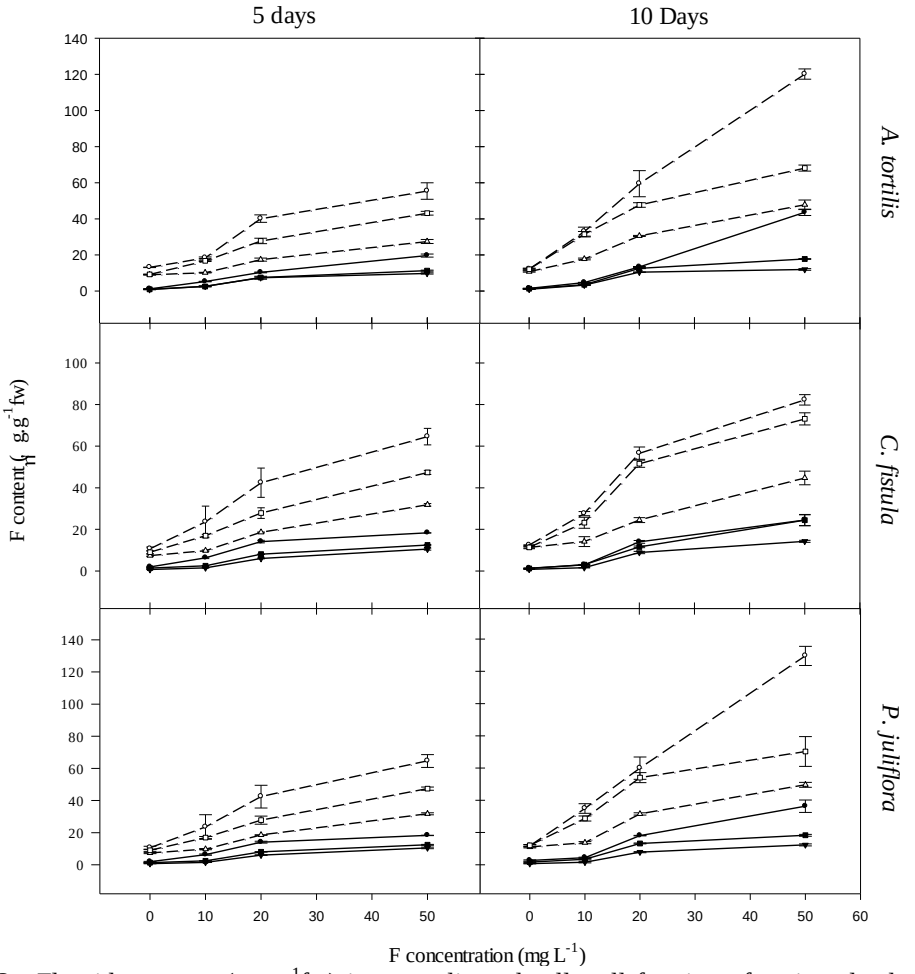

Fig. 3. Fluoride content (µg g^{-1}fw) in cytosolic and cell wall fraction of various land plants grown in soilrite in different concentrations of F for 5 and 10 days. Root cell wall F (—●—), root cytosol F (— -○- —), stem cell wall F (—▼—), stem cytosol F (— -△- —), leaves cell wall F (—■—) and leaves cytosol F (— -□- —).

In *A. tortilis*, a gradual rise in cell wall and cytosolic F with increase in its concentration in the nutrient medium was observed. Among all three organs, *viz*. roots, stem and leaves, maximum increase was seen in roots (30% in cell wall at 10 days). The cytosol accumulated maximum F, but a steep rise in F content at 50 mg L^{-1} as compared to control (30 folds at 10 days) was observed in cell wall fraction of roots. *C. fistula* plants had a similar trend of F accumulation as in *A. tortilis* plants. The highest increase (*ca.* 22%) as compared to control was seen in cell wall fraction of roots at 5 days. *P. juliflora* plants accumulated maximum F in its all organs and thus had highest F

content in both cell wall and cytosolic fraction. For example, the F content in root cell wall at 10 days was 161.76 µg g^{-1}fw, which was highest among all plants.

The F content in the cell wall and cytosolic fraction of the three plant species grown in soilrite is shown in Fig.3. Organwise studies made in the preceding section suggested that soilrite-grown plants accumulate less F than hydroponically grown ones. This was reflected in comparatively lesser F content in the cell wall and cytosolic fraction of these plants.

Hence roots accumulated maximum F in their cell wall and cytosol and thus showed a steep rise in F content (from control to 50 mg kg^{-1} F treatment). However, this was not observed in stem or leaves. The reason could be low mobility of F in the plant, as it has relatively low permeability through the endodermis (Cooke *et al*., 1978; Keller, 1980; Takmaz-Nisancioglu and Davison, 1988). It is thought that the endodermis acts as a barrier to entry into conducting system, which limits transport to the shoot. F reaches the vascular system by a non-selective root that by-pass the endodermis (Pitman, 1982) and the concentration in individual leaves may be a function of concentration in the rooting medium and of water flow. Thermodynamically, increased soil acidity results in greater F bioavailability and hence, greater plant uptake (Horner and Bell, 1995), while increasing bioavailable Ca results in lower leaf F content but increased root F content. It is conjectured that high root F is associated with the formation of CaF_2 either outside or inside the root (Ramagopal *et al*., 1969). F absorbed by roots is transported to shoots through transpiration stream and accumulates mostly in leaf tissues. Stem just acts as a transport medium, and hence is least preferred organ of F accumulation.

High F levels in organs of hydroponically grown plants might be associated with the higher uptake of F compared with the soil-cultured plants. Because of the complex chemistry of soil, it is likely that free F ions from outside sources are complexed by the soil and hence very little F is available for uptake by plants. In Hoagland's nutrient media, more free F ions are available for uptake by plants in culture. That high activities of F in solution did not affect the moisture content in roots, stem and leaves suggesting that these plant species are able to tolerate high concentrations of F probably by detoxifying F at the cellular level in the plants.

It was quite early (Ledbetter *et al*., 1960) that fractionation studies on F exposed tomato plants revealed that F was found in decreasing order of accumulation in supernatant, cell wall, chloroplast, water-soluble proteins and mitochondria. After some years, Chang and Thompson (1966) found that the chloroplasts were the site of highest F accumulation. This opinion holds well till date. In view of the fact that the present study only determines the F content

in two major cellular fractions *viz.* cell wall and cytosol, our data supports the findings of above workers.

Concluding Remarks

Among all plants studied, *P. juliflora* accumulated maximum F and *A. senegal* the minimum. The increase in the F accumulation (dry weight basis) after 10 days of treatment was in the order of *A. senegal* < *A. lebbeck* < *P. cineraria* < *A. nilotica* < *A. tortilis* < *A. indica* < *C. fistula* < *P. juliflora.*

The organwise F distribution in study plants revealed that roots accumulated maximum F. Organwise studies also showed that the soilrite grown plants accumulated less F than hydroponically grown. In general, cytosolic fraction accumulated more F in comparison to cell wall.

The present study explores a new area of phytoremediation of F where *P. juliflora* can be of potential use. Suitability of a plant for phytoremediation could be determined by its ability to produce a high aboveground biomass and high bioconcentration and transfer factors (Asada *et al*., 2006; Zabłudowska *et al*., 2009). *P. juliflora* has to fulfill all of these characteristics and it should be able grow in the presence of other toxic metals. Further studies are in offing to establish the phytoremediation potential of this plant in the field.

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