FLUORIDE REMOVAL FROM WATER BY PLANT SPECIES THAT ARE TOLERANT AND HIGHLY TOLERANT TO HYDROGEN FLUORIDE

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SUMMARY: Seventeen plant species that are tolerant and highly tolerant to HF were selected to establish hydroponic cultures. These species were exposed to 2.5, 4, 5, and 10 mg fluoride (F) ion/L to identify plants with the capacity to remove F from water and might be useful for phytoremediation of F. Only *Camellia japonica, Pittosporum tobira*, and *Saccharum officinarum* were able to remove F from water with some degree of efficiency. At 4 mg F/L, sugar cane (*Saccharum officinarum*) removed 40% of F compared to the 7.5% and 15% removed by *C. japonica* and *P. tobira*, respectively. A concentration of 5 mg F/L seemed to be the maximum level tolerated by these plant species. The potential use of *S. officinarum* for remediation of F-contaminated water is discussed.

Keywords: *Camellia japonica*; Hydroponic cultures; Phytoremediation; *Pittosporum tobira*; Sugar cane; *Saccharum officinarum*.

INTRODUCTION

Phytoremediation is an expanding technology that employs higher plants for the cleanup of contaminated environments that has several advantages over physical remediation methods, including lower cost. The conventional methods of remediation may cost anywhere from US $10 to $1000 per cubic meter, while the cost of phytoextraction is estimated to be as low as $0.05 per cubic meter.[1-2] This technology can be applied to both organic, and inorganic pollutants present in soil, water, or air. Plants can remove a variety of pollutants including pesticides,[3,4] antibiotics,[5,6] metals,[7-8] and aromatic compounds.[9-10] Plants have also been found to accumulate ionic fluoride (F) in their tissues. Solution levels of 1.5 to 4 mM F may lead to dry mass concentrations of 70 to >300 mg F/kg dry mass in shoots, and more than 3000 mg F/kg in roots.[11-13] However, few investigations have been reported for F removal from water, or soil by plants.[14-16] Plant sensitivity to F is highly variable and depends on species, as well as the timing, duration, and level of exposure.[17,18] Plant species tolerant to HF in air have been reported to grow at levels up to 30 µg HF/m^3, whereas sensitive plant species generally show damage at 0.4 to 1.0 µg/m^3 after several days of exposure to hydrogen fluoride (HF).[19] Plants tolerant to HF must have cellular mechanisms to avoid or reduce toxic effects of F and therefore represent good candidates for their potential to phytoremediate water containing high levels of F.

In this work, we selected species tolerant and highly tolerant to HF to establish hydroponic cultures. These plant cultures were then exposed to different F concentrations in order to identify those with a putative capacity to remove F from water, and to examine the kinetics of removal.

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MATERIALS AND METHODS

Plant material: Selected for the study were plants considered tolerant (T) or highly tolerant (HT) to HF according to tables of relative sensitivity (Table 1).[19,20]

Table 1. Species selected for the establishment of hydroponic cultures

Species	Category[a]
Camellia japonica	HT
Chaenomeles lagenaria	HT
Cyclamen persicum	HT
Dianthus barbatus	HT
Ligustrum vulgare	HT
Lonicera japonica cv. Halliana	HT
Malva sylvestris	HT
Medicago sativa	T
Olea europaea	HT
Pelargonium L.	T
Pyracantha coccinea	HT
Pittosporum tobira	T
Robinia pseudoacacia	HT
Saccharum officinarum	T
Salix pentandra	HT
Salvia splendens	HT
Ulmus pumila	T

[a]HT: highly tolerant to HF; T: tolerant to HF.

The plants were kindly donated by the commercial nursery Savia Forestal in the city of San Luis Potosí, excepting the species *Saccharum officinarum* which was provided by the sugar mill Central Motzorongo SA de CV, located in Veracruz State, México.

Establishment of hydroponic cultures: For the establishment of hydroponic cultures, plastic containers previously treated with 10% HNO_3 and rinsed three times with deionized water were used. The container vessels (8-cm deep) were covered with plastic caps (with a perforation to place plants) to avoid evaporation, and with dark plastic bags to avoid the incidence of light on the hydroponic solution. Half concentration of Murashige and Skoog medium[21] (MS) containing micro and macronutrients, but without sucrose (pH 5.7) was employed as nutrient medium. Plant species ca. 20-cm in length were transferred to the container vessels, and kept in greenhouse conditions (18–30°C, relative humidity 68.8%, light 127 μmol/m^2/s). The solutions were aerated using polyvinyl chloride manifolds fed from a commercial air pump. Six individual seedlings from each species were set up for study.

Fluoride removal experiments: A stock solution was prepared by dissolving 221 mg NaF in 1.00 L of deionized water (100 mg F/L). Appropriate dilutions were made to give 2.5, 4, 5, and 10 mg F ion/L.

Plants adapted to hydroponic conditions were transferred to plastic vessels containing 150 mL of the MS medium containing 2.5, 4, 5, or 10 mg F/L during different periods of time. Control plants were cultivated under the same conditions without NaF in the solution. Five mL of medium were collected at 0, 7, 14, 18, and 21 days, and the F concentration was measured using an ion selective electrode (960900, Thermo Orion) with F solutions of known concentration for calibration.

RESULTS

Eight of the seventeen species tested adapted well to hydroponic conditions after 21 days (Table 2).

Table 2. Fluoride effects and removal by hydroponic plants in 4 mg F/L culture media

Species	New shoots	Wilting (%)	Oxidation (%)		Chlorosis (%)			F removed by day 21 (mg/L)
	21 d	21 d	6 d	21 d	6 d	12 d	21 d	
Camellia japonica	0	0	20	30	0	0	0	0.3
Chaenomeles lagenaria	0	30	60	100	0	0	0	0
Cyclamen persicum	0	0	0	0	0	0	0	0
Dianthus barbatus	0	0	0	0	0	0	0	0
Olea europaea	0	0	0	0	0	0	0	0
Pelargonium L	1	0	0	0	5	20	25	0
Pittosporum tobira	0	0	0	0	0	0	0	0.6
Saccharum officinarum	0	0	0	0	0	0	0	1.3

These species were selected to conduct F removal experiments as an initial approach to select plants tolerant to F that could be of use in phytoremediation. As seen in Table 2, 4 mg F/L was found to induce different toxic effects on plants, such as chlorosis in *Pelargonium,* necrosis in *C. lagenaria*, and moderate oxidation in *C. japonica*, but it had no apparent toxic effects on *C. persicum, D. barbatus, O. europaea, P. tobira,* and *S. officinarum*. However, only *C. japonica, P. tobira*, and *S. officinarum* were able to remove F from the medium to any measurable degree.

As seen in Figure 1, among the three species selected for the study of the kinetics of F removal, *C. japonica* seedlings presented a progressive F uptake until day 7 when the maximum removal (0.3 mg F/L) was reached. F uptake by the *P. tobira* cultures was faster, however, and twice as efficient (0.6 mg/L), while *S. officinarum* continued accumulating F (1.6 mg/L) until day 21 without apparent saturation.

To test whether tolerance was dependent on F concentration, plants were exposed to different concentrations of F. The results showed that wilting of plants was species-dependent, and proportional to time of exposure, and concentration (Figure 2).

In regard to F uptake, *C. japonica* plants exposed to 2.5 mg F/L presented the same kinetics shown in Figure 2 (data not shown). Experiments using 5 and 10 mg F/L were not performed because the plants did not have a healthy appearance. *P. tobira* (Figure 3) and *S. officinarum* (Figure 4) plants removed the anion faster at the 5 mg F/L level, in comparison to the 2.5 mg/L level. At 10 mg F/L, F removal was not observed suggesting that once a threshold is reached, toxicity to the plant became severe by exceeding the mechanisms of tolerance.

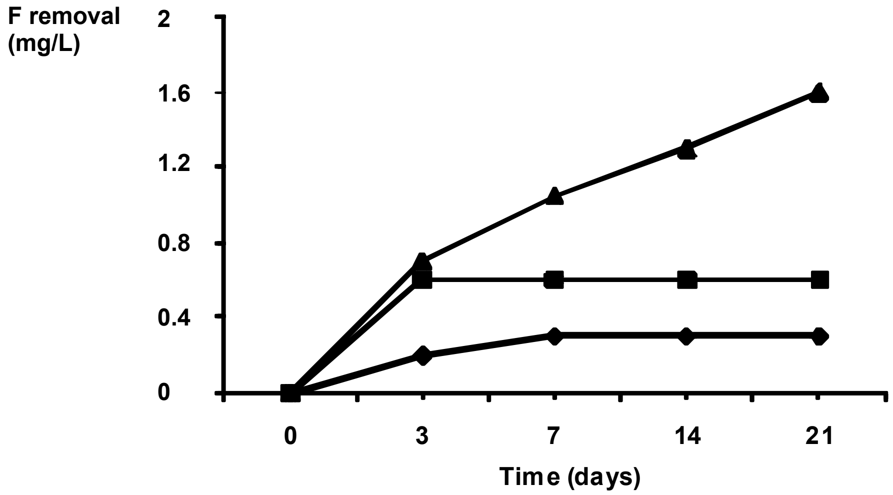

Figure 1. F removal by hydroponic cultures of *Camellia japonica* (◆), *Pittosporum tobira* (■), and *Saccharum officinarum* (▲). Seedlings were maintained in culture media containing 4 mg F/L.

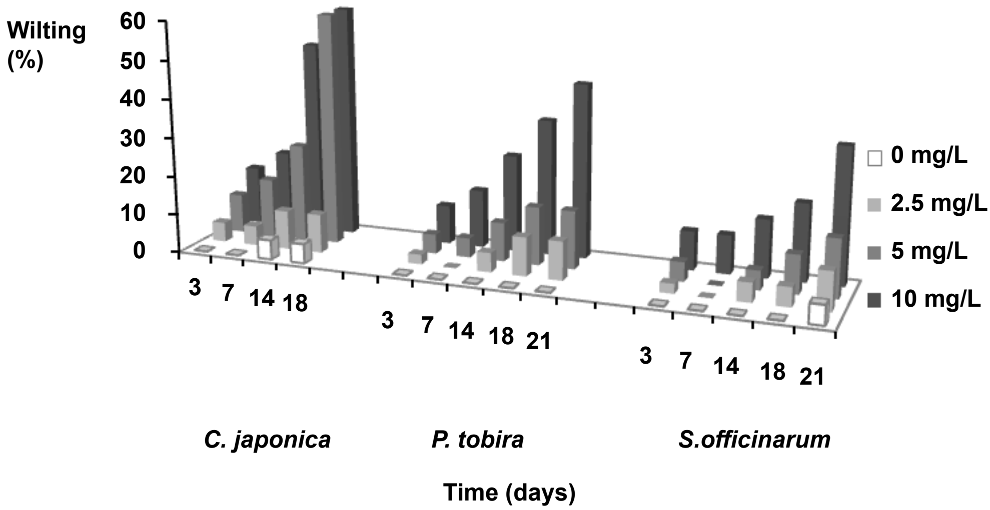

Figure 2. Wilting effect of different F concentrations and duration of exposure on *Camellia japonica*, *Pittosporum tobira*, and *Saccharum officinarum.*

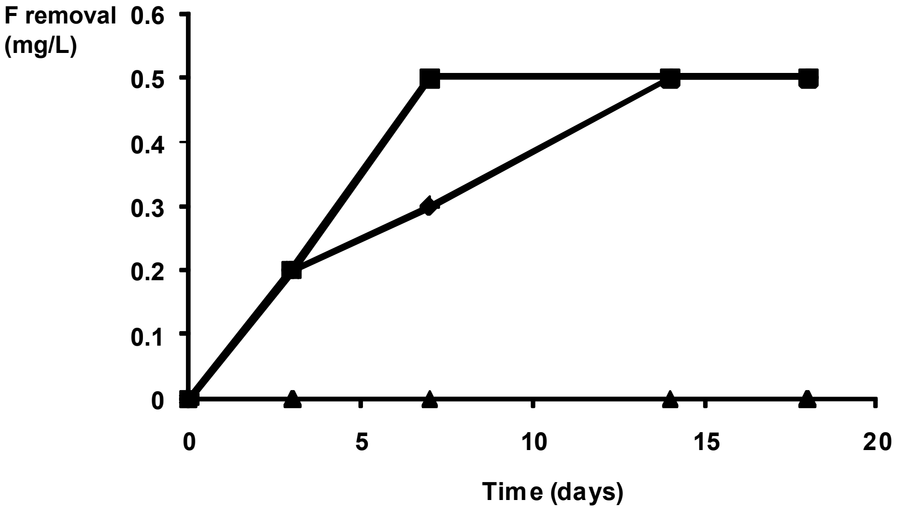

Figure 3. F removal by *Pittosporum tobira* exposed to 2.5 (◆), 5 (■), and 10 (▲) mg F/L.

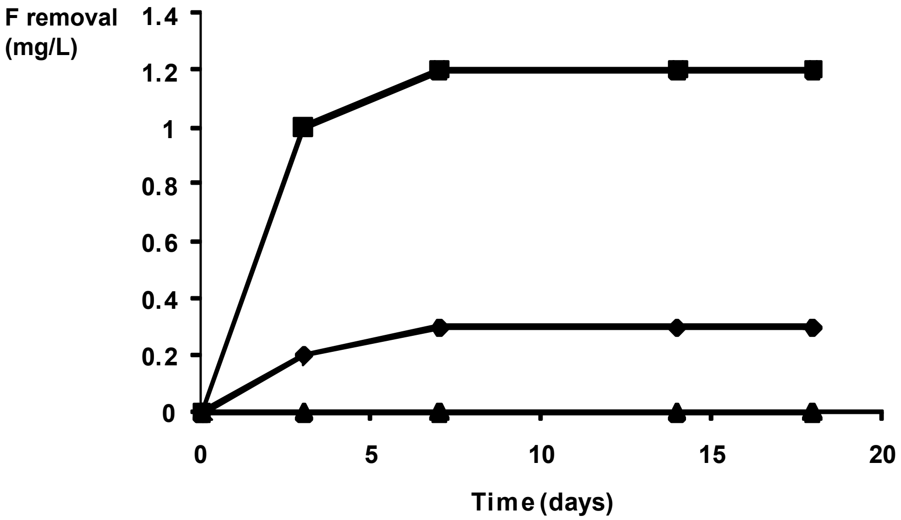

Figure 4. F removal by *Saccharum officinarum* exposed to 2.5 (◆), 5 (■), and 10 (▲) mg F/L.

DISCUSSION

In this study, we have identified three plants sufficiently tolerant to HF have the capacity to remove F from water: *C. japonica, P. tobira*, and *S. officinarum.* Two characteristics are important for the use of plants for phytoremediation: good phytoremediation capacity, and high production of biomass.[1,2] *S. officinarum* (sugar cane) was twice and five-fold more efficient in removing F from water than *P. tobira,* and *C. japonica*, respectively. In the presence of 4 mg F/L, sugar cane plants 20 cm tall, removed 40% F present in the culture medium, compared to the 7.5%, and 15% removal by *C. japonica* and *P. tobira*, respectively. For *S. officinarum*, F uptake did not show any apparent saturation at 21 days (Figure 1), indicating that higher removal could be possible at longer exposure times. In addition, uptake can be improved using bigger plants. *S. officinarum* is a species with one of the highest growth rates. A stem of sugar cane grows to a height of 4 or 5 meters in 11 to 18 months. These growth rates represent a 20 to 25 fold higher biomass compared to the other plants examined in this study.

At 5 mg F/L, incipient wilting symptoms appeared and a maximum uptake of F was observed in *P. tobira*, and *S. officinarum*, suggesting that this F concentration is the maximum level that these species can tolerate.

In central and northern Mexico are extensive areas of endemic fluorosis affecting about five million people.[23] However, F ion concentrations above 5 mg/L are quite rare. Also, in Kenya[24] and in India,[25] a large percentage of groundwater samples exceed 1.5 mg F/L, but relatively fewer wells contain more than 5 mg F/L. Therefore, the use of *S. officinarum* could be an alternative for at least partial remediation of water with concentrations ≤ 5 mg F/L in these countries.

The greater removal of F from the 5 mg F/L culture medium by *S. officinarum* compared to *C. japonica* and *P. tobira*, might be explained in terms of an activation of the detoxification processes. It has been proposed that tolerant plants may deactivate, or sequester F by reaction of F with calcium present in cell walls to form insoluble CaF_2.[13,19] Sequestration of the anion in the vacuole could be another possible mechanism of tolerance. Further studies will be required to determine the specific mechanism(s) of tolerance by *S. officinarum* to F.

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