REVIEW

Fluorine: a biohazardous agent for plants and phytoremediation strategies for its removal from the environment

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Abstract

Fluorine, one of the most abundant elements found on earth, acts as an environmental xenobiotics even at sparingly low concentrations. Uncontrolled anthropogenic activities have steeply increased the F content in the air, water, and soil. Irrigation of crops and vegetables with F contaminated groundwater or agricultural practices in contaminated soils adversely affect their physiological and biochemical parameters, leading to inhibited growth and productivity. Some plants can translocate the toxic ions from roots to shoots and accumulate them in the edible parts. Bioaccumulations of F have hazardous outcomes, as their concentrations in edible parts can be higher than the safe value. Screening of F hyperaccumulators and F-tolerant plants has been performed to facilitate phytoremediation. Some plant bioindicators have been identified which can be used to analyse the extent of atmospheric F pollution. Cumulative use of these organisms through proper scientific planning programs can potentially improve the agricultural soil quality in terms of their toxic F content. Future research should focus on proper execution of these phytoremediative strategies *via* robust field trials. High throughput genetic analyses should also be performed to identify quantitative trait loci which can be exploited to generate F-tolerant characters in susceptible crop cultivars.

Additional key words: chlorophyll, F bioindicators, F hyperaccumulators, growth, photosynthesis, xenobiotics.

Introduction

The earth's crust contains about 950 mg(F) kg^{-1}(soil). It is the 13th most abundant element in nature and is stored as Fs in naturally occurring minerals like fluorspar, cryolite, fluorapatite, *etc*. The extremely high electronegativity accounts for the high F reactivity (Hong *et al.* 2016). Low doses of NaF ranging between 0.3 - 1.0 mg dm^{-3} are often introduced in drinking water by the municipalities to prevent dental caries and osteoporosis. However, ingestion of F beyond the safe limit of 1.5 mg dm^{-3} (proposed by the World Health Organization, WHO) results in irreversible damage, e.g., skeletal deformities, non-skeletal fluorosis, and secondary neurological complications in animals as well as in humans (Choubisa 2013).

F contamination exerts hazardous effects on the ecosystem. Plants can either take up F from the contami-nated soil *via* roots or can absorb F from the atmosphere (Bhat *et al.* 2015). F released into the atmosphere from anthropogenic sources; it hydrolyzes into corrosive hydrogen F (HF), which reacts with materials in aerosols and vapour to form non-volatile stable Fs (Hong *et al.* 2016). High content of atmospheric F, reaching a maximum value of 2 mg kg^{-1}(total air) was reported during atmospheric precipitation in Poland (Walna *et al.* 2013). It has been seen that HF content as low as 1 µg kg^{-1}(total air) can initiate injuries in the sensitive plants (Gupta *et al.* 2009). A massive F deposition of 52 mg m^{-2}(soil) year^{-1} was detected during a two-year study in Poland (Walna *et al.* 2013).

Even exposure to low amounts of F is sufficient to cause physiological complications like necrotic lesions, burning, chlorosis, leaf damage, and inhibition in

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development and reproductive capability in the sensitive plants. The stress-associated symptoms in plants are observed gradually and more prominently on prolonged exposure to F (Hong *et al.* 2016). F bioaccumulation in the edible plant parts (Mondal and Gupta 2015) is a problem of global interest because different countries across Asia, Africa, Australia, and South America are facing acute endemic fluorosis (Susheela 1999, Meenakshi and Maheshwari 2006).

Though a significant amount of research has been conducted on multiple abiotic stresses like salinity, drought, cold, heat, light and heavy metal toxicity (Banerjee and Roychoudhury 2017, 2018a), less literature is available on the effects of this 'slow poison' on plants. In this review, we have thoroughly discussed the adverse effects of F as a biohazardous agent as well as highlighted the prospects of F phytoremediation.

F translocation from soil to plants

Physico-chemical properties of fluorides in the soil: According to Susheela (1999), F content in water have been recorded as high as 48 mg dm^{-3} across different states of India. High F content has been detected in rivers (12 - 26 mg dm^{-3}), springs (15-63 mg dm^{-3}) and alkaline ponds and lakes (60 - 690 mg dm^{-3}) in northern Tanzania (Nanyaro *et al.* 1984). Farooqi *et al.* (2007) reported that out of the 147 groundwater samples tested in Pakistan, 75 % largely exceed the F safe limit (1.5 mg dm^{-3}) and this concentration is toxic not only for animals and humans, but also affected plants. It has been seen that extremely contaminated (often heavy clay) soil can reach content of Fs 1 000 - 3 500 mg kg^{-1}(d.m.) (Hong *et al.* 2016) but the normal soil F content can range between 150 - 400 mg kg^{-1}(d.m.) (Bhat *et al.* 2015). However, the critical value of soil F beyond which F uptake by plants and translocation to shoots increased species-specific.

F is a ubiquitous toxin which mostly remains adsorbed to clays and oxy-hydroxides with a very low percentage dissolved in the soil solution (Marier 1971). Therefore, F mobility varies with the soil permeability and sorption capacity. The sorption capacity is dependent on soil pH, types of sorbents and soil salinity (Tylenda 2011). Due to the formation of stable bonds with $Fe^{2+/3+}$, Al^{3+}, Mg^{2+} and Ca^{2+}, F content is much higher in the silt, clay loam and non-calcareous (containing high Al^{3+}) soil than in sandy soil (Hong *et al.* 2016). Soils with high Ca^{2+} content are effective in fixing F in the form CaF_2 (Abugri 2010). As a result, a small percentage of F^- exists in free form in soil containing high Al (Vasudevan *et al.* 2003). Thus, soil need to be alkaline in order to contain high doses of free F^-. This aids in the release of F from the soil surface resulting in enhanced plant bioavailability. Again, alkaline pH reduces soil F retention. This is due to the formation of electrostatic potential and displacement of bound F^- by OH^- (Saxena and Rani 2012). Therefore, F bioavailability for plants is greatly dependent on the pH and physico-chemical properties of the soil. Formation of AlF_x under acidic conditions increases water solubility of F, whereas alkaline conditions promote desorption of free F (Wehr *et al.* 2014) (Fig. 1).

Fluoride entry into plants: Plants absorb F from air, water, and soils. However, the amount of F uptake varies with the type of plant, soil characteristics, and the amount of F in the air, soil, or water used for irrigation (Anshumali 2014). F content in the tissues of *Triticum aestivum* was analysed by a potentiometric method using a F-selective electrode. Different content of F was observed in the stems, leaves, and roots (Agrawal and Chauhan 2014). Variable F accumulation in the shoot biomass has been reported even among cultivars. This is because, in spite of passive uptake of F by roots, the variable root-shoot translocation efficiency plays a crucial role in determining F accumulation in shoots (Mondal 2017).

The Ca^{2+} content in the root cell wall primarily determine F sensitivity. This is because F^- is scavenged by Ca^{2+} present in the root cell wall. Thus, species with high Ca^{2+} in this region better control the entry F^-. It has been hypothesized that F^- is absorbed through chloride channels and chloride deficiency enhances F^- uptake (Baunthiyal and Ranghar 2013). Aquaporins might also be involved in F transport (Baunthiyal and Ranghar 2013). Due to the high electronegativity of F, it is quite obvious that symplastic transport is not permitted. The fixed negative charge of the plasma membrane excludes the permeability of F ions. Hence, apoplastic transport *via* the cell wall and intercellular spaces is common (Agrawal and Chauhan 2014) (Fig. 1). The endodermis acts as a protective barrier and prevents effective F transport to the vascular tissues, thereby minimizing the root-to-shoot translocation. This is in line with the observation of Jha *et al.* (2009) who showed that the order of F retention in *Allium cepa* is roots > shoots > bulbs. Thus, the F that reaches the shoots is transported through a non-selective route. This route might bypass the endodermis *via* an unknown mechanism (Hong *et al.* 2016). The xylem sap flow carries F to the leaves (Panda 2015). The F accumulation in the shoots varies across species and is a function of F concentration in the rooting medium and the rate of water flow (Pitman 1985). In a recent research, Chen *et al.* (2017) found synergistic negative effect of F and Cd on the growth of radish plants. Both F and Cd accumulation could be detected within the leaves and at the leaf tips (Chen *et al.* 2017).
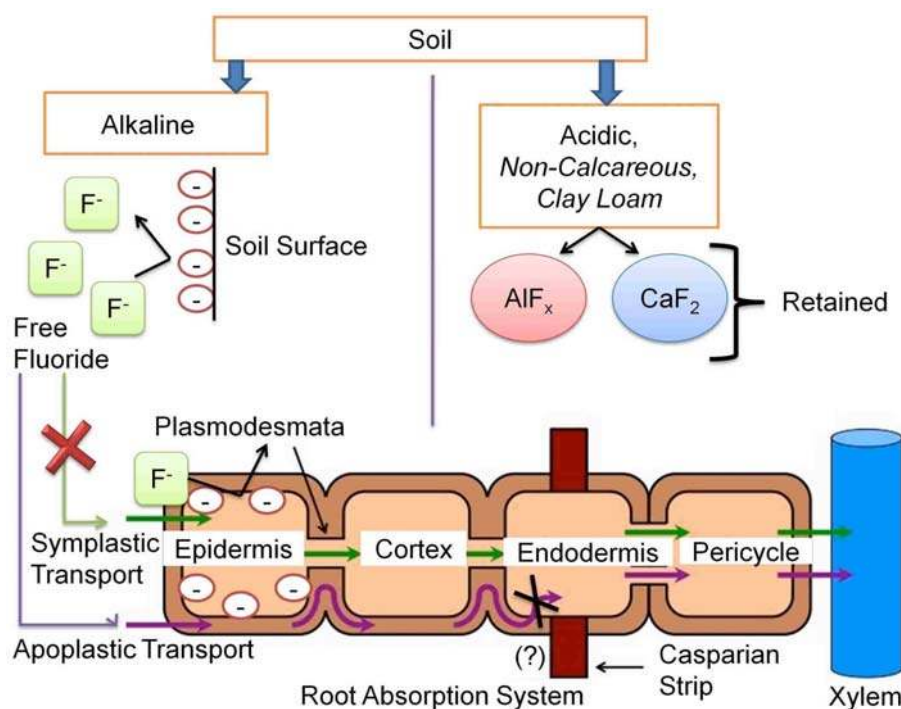
Fig. 1. F is retained as aluminium F complexes (AlF_x) and calcium F (CaF_2) in non-calcareous, clay loam soils at acidic pH and is unavailable to the plant roots. Under alkaline conditions, the bioavailability of free F increases due to repulsion of negatively charged F ions with the negative soil surfaces. The free F is absorbed *via* chloride transporters or aquaporins in the plant roots and it is transported through the apoplastic route. Due to strong repulsions between F and the negatively charged plasma membrane, transport *via* the symplastic route is less common. The endodermis acts as a barrier and prevents F ions to progress towards the pericycle. F transport towards the vascular tissues from this point is not well characterized (?) and possibly occurs through a non-selective route bypassing the endodermis.

Fluoride phytotoxicity

Morphological and anatomical symptoms: A cell suspension of *Arabidopsis thaliana* treated with 20 mM NaF for 2 h exhibited no loss of cell viability. However, cellular development was strictly inhibited by exposure to even 1 mM NaF for 10 d (Pulyaevskaya *et al.* 2011). Fs can accumulate in the plant foliage and typical injury is marginal and tip necrosis gradually spread inward. F is carried and deposited in the leaf edges by the vascular tissues (Fig. 2). Depending on the content in the cell sap, F firstly damages the spongy mesophyll and lower epidermis and then chloroplasts in the palisade cells and the upper epidermis (Panda 2015). Members of *Pinaceae* exhibit necrotic patches which spread from the needle tip to the base (Hong *et al.* 2016). More detail information concerning F-induced damages in different plant species are in Table 1 Suppl.

F stress and initial development in plants: Proper seed germination and seedling growth are crucial for plant survival. F interferes with active metabolism and lowers the rate of cell division and expansion. This inhibits germination and early seedling development (Ram *et al.* 2014). Seed germination is also affected due to F-induced degeneration of gibberellic acid (GA) in the aleurone,

leading to the abnormal metabolism of endosperm saccharides (Gadi *et al.* 2016). Inappropriate seedling development and unbalanced nutrient uptake due to F interference reduced shoot and root length, fresh and dry masses and vigour index in *Oryza sativa, Triticum aestivum, Cyamopsis tetragonoloba, Cicer arietinum, Citrullus lanatus*, and *Abelmoschus esculentus* (Gupta *et al.* 2009, Datta *et al.* 2012, Ram *et al.* 2014, Gadi *et al.* 2016, Iram and Khan 2016).

Impact of fkuorides on photosynthesis: The Ca^{2+} is a crucial secondary messenger which participates in multiple signalling cascades in the plant system (Roychoudhury and Banerjee 2017). F chemically interacts with Ca^{2+} and precipitates as CaF_2 to alter membrane permeability (Stevens *et al.* 1998). Calcium dependent protein kinases (CDPKs) act as signalling hubs in multiple abiotic stress responses (Roychoudhury and Banerjee 2017). Lowering of intracellular Ca^{2+} content can thus disrupt the abiotic stress-responsive signalling process during F stress (Fig. 2).

Fs act as metabolic inhibitors in plants (Iram and Khan 2016). Prolonged F stress induces chlorosis and deteriorates basic physiological processes like

photosynthesis. F strongly associates with Mg^{2+} and $Fe^{2+/3+}$ and decreases their upward translocation to the leaves (Elloumi *et al.* 2005). This inhibits the biosynthesis of chlorophylls (Chls) and carotenoids (Cars) (Table 1). The participation of γ-aminolevulinic acid in the Chl biosynthetic pathway is also prevented during F stress (Wallis *et al.* 1974). The MgF^+ complex can degrade plant pigments (Abdallah *et al.* 2006). Content of Chl *a* and *b* significantly decreased in *Oryza sativa* and *Triticum aestivum* exposed to F stress (Gupta *et al.* 2009, Bhargava and Bhardwaj 2010). Chakrabarti and Patra (2015) showed Chl and Car degradation in two *indica* rice cultivars exposed to 10, 20, and 30 mg dm^{-3} NaF. Similarly, Mondal (2017) detected pigment degradation in four *indica* rice cultivars exposed to 5, 10, and 20 mg dm^{-3} NaF. Severe chlorosis and necrosis were seen on the young leaves of *Populus deltoides* irrigated with water containing 500 mg dm^{-3} of F for 100 d (Singh and Verma 2013). Antho-cyanins have antioxidant properties and have been associated with stress tolerance (Paul *et al.* 2017). *Salicornia brachiata* plants exposed to F for 100 d exhibited high anthocyanin content and decrease in content of Chls and Cars (Reddy and Kaur 2008). Radish plants growing on soils co-contaminated with F and Cd exhibited high absorption of Zn^{2+} and Mn^{2+}. However, Cu^{2+} absorption was drastically reduced

(Chen *et al.* 2017). Since these micronutrients often act as co-factors of essential enzymes, their altered profile influence important signalling pathways associated with the growth and development of the plant (Banerjee and Roychoudhury 2018b).

Severe chlorosis induced by F stress detrimentally affects photosynthesis (Mondal 2017, Banerjee and Roychoudhury 2018c). Activities of the enzymes associated with Calvin cycle are reduced. The main enzymes affected by F exposure are chloroplast ATPase, ribulose 1,5 bisphosphate carboxylase/oxygenase, and sucrose synthetase (Baunthiyal and Ranghara 2014). F-induced stress impaired the stomatal conductance resulting in limited CO_2 diffusion from the atmosphere into the cell and reduced photosynthetic CO_2 assimilation (Singh *et al.* 2013). Singh and Verma (2013) determined Chl fluorescence and showed reduced photosynthetic efficiency (F_v/F_m) in poplar plants exposed to F stress. The chlorophyll fluorescence yield was monitored by plant efficiency analyzer. The decrease in photosysterm (PS) II occurs due to distinct decrease in the activity of chlorophyll binding proteins (CP43 and CP47) of core antenna subunits of PS II as affected by F treatment, along with increase in PS I. Effects of F on photosynthesis are pronounced (Table 1 Suppl.), but the physiological basis of such deterioration is still required
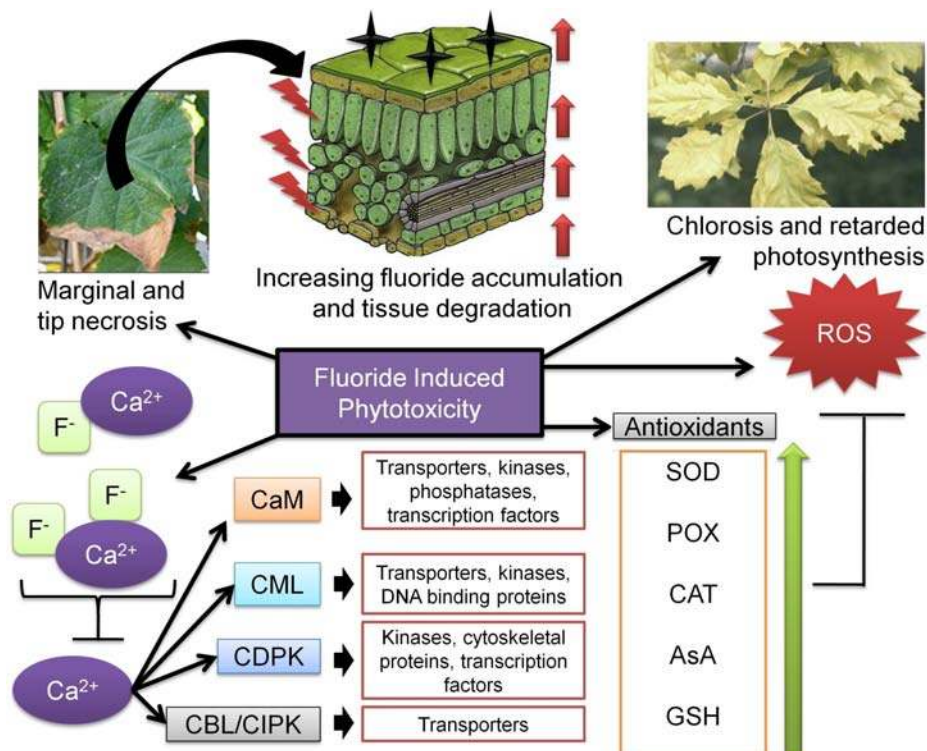

Fig. 2. Accumulation of F triggers marginal and tip necrosis in leaves accompanied by degradation of mesophyll tissues. Gradual increase of F content firstly affects lower epidermis, then spongy mesophyll, palisade parenchyma, and finally the upper epidermis. The anatomical deterioration is also associated with physiological abnormalities like chlorosis and retarded photosynthesis. F also chelates calcium (Ca^{2+}) ions and hinders downstream signalling pathways. Ca^{2+} dependent molecular signaling components like calmodulin (CaM), CaM-like proteins (CML), calcium dependent protein kinase (CDPK), calcineurin B-like protein (CBL)/CBL-interacting protein kinase (CIPK), *etc.*, are inactivated. F also induces oxidative damages by triggering the accumulation of ROS. This activates the antioxidant machinery to protect the plant.

to be elucidated. One possibility of indirect effects is F- induced water stress as low relative water content (RWC) was observed in plants like *O. sativa, T. aestivum, Brassica oleracea, B. juncea, Beta vulgaris, Apium graveolens, Daucus carota, Allium sativum, Pisum sativum, Cucumis sativa*, and *Lens culinaris* irrigated with F contaminated water (Mondal and Gupta 2015) (Table 1 Suppl.). Such low RWC also inhibits normal growth.

Biochemical alterations induced by Fs: Biochemical parameters are important indices for determining plant sensitivity against a particular stress. F stress suppresses respiration up to 50 % by inhibiting activities of mitochondrial and glycolytic enzymes like succinate dehydrogenase, malate dehydrogenase, and enolase (Miller 1993). F tends to accumulate in the cellular organelles with alkaline pH like mitochondria and chloroplasts and inhibit the acid phosphatase and membrane ATPase activities (Struglics *et al.* 2000). The AlF_4^- complex is a phosphate ion analogue and so it can inhibit P transport. Such fluoride-mediated inhibition in P transport has been observed in the membrane proteins of potato tubers exposed to elevated content of F (Golicnik 2010, Struglics *et al.* 2000). Inhibition of phosphorylation directly prohibits processes like glycolysis, nucleotide synthesis, signalling, *etc.* (Barbier *et al.* 2010). Mondal (2017) reported significant lipid peroxidation and root ion leakage in four indica rice cultivars exposed to increasing concentrations of F.

Amino acids and total nitrogen metabolism are also affected in plants exposed to F. An increase in free amino acid content due to degradation of the soluble proteins was observed in the leaves of tea plants exposed to F stress. The leaves accumulated high content of asparagine, aspartic acid, serine, glutamic acid, and pipecolic acid (Cai *et al.* 2016). An interesting correlation between nitrogen metabolism and F stress has been observed among F tolerant and hypersensitive plants (Szostek and Ciecko 2017). It was seen that plants with variable sensitivities to F stress accumulated variable content of total N in the shoot biomass in a species-specific manner (Table 1 Suppl.). *Zea mays*, being F tolerant, possibly has developed an evolutionary strategy of preventing F-induced protein degradation and hence the stressed plant does not require up regulation of protein synthesis and low N content could be detected (Szostek and Ciecko 2017). On the contrary, in spite of being F tolerant, *Lupinus luteus* plants accumulated high amount of total N in their shoots (Szostek and Ciecko 2017). Thus, these plants exhibit F tolerance by increasing protein synthesis and tilting the equilibrium away from protein degradation. F hypersensitive plants like *Lupinus angustifolius* has been found to record high N content in the shoots due to unregulated protein degradation (Table 1 Suppl.).

F stress deteriorates nitrogen assimilation by inhibiting nitrate reductase, the rate limiting enzyme of nitrogen acquisition (Rao *et al.* 2013). The F-induced decline in the total protein content can be explained by channelizing the degraded products towards metabolic processes for obtaining energy and tackling stress (Szostek and Ciecko 2017). Mulberry plants grown under F stress showed a 59 % decrease in leaf protein content (Rao *et al.* 2013). Pal *et al.* (2012) reported decreases in total protein content in 13 plant species irrigated with F contaminated water. *Abelmoschus esculentus* seedlings irrigated in F contaminated water, exhibited reduced percentages of nitrogen and protein compared to the control plants (Iram and Khan 2016). However, potato plants grown in F contaminated soil exhibited an increase in total protein content possibly to counterbalance the protein loss (Saleem *et al.* 2015).

F stress decreases the water potential by inhibiting water uptake and the water conductivity of the roots. As a result, the osmotic potential increases (Gadi *et al.* 2016). Under such conditions, plants tend to accumulate inorganic ions, soluble sugars, and compatible solutes (osmoprotectants) to maintain the cellular hydration (Paul *et al.* 2017). Increase in the content of compatible solutes like amino acids, proline (Pro), and phenols have been reported (Table 1 Suppl.). Compatible solutes also protect the membrane systems and stabilize protein and enzymatic structures. Pro has even been associated with post-stress recovery and the generation of stress memory *via* epigenetic regulations during salt stress (Roychoudhury *et al.* 2015). Remarkable accumulation of soluble sugars has been observed in several plant species exposed to F stress (Table 1 suppl.). They act as osmotica to facilitate root water transport and stabilize proteins and membrane structures (Ram *et al.* 2014, Banerjee and Roychoudhury 2016). Elloumi *et al.* (2005) observed steady accumulation of nonreducing sugars in plants grown under F stress.

F stress and the antioxidant system: The discussions in the previous sections altogether conclude that F severely retards the physiological parameters in sensitive plants. Such inhibitions might be due to unregulated production of reactive oxygen species (ROS) like hydrogen peroxide, hydroxyl radica, and superoxide radical (Banerjee and Roychoudhury 2018d) (Fig. 2). The ROS non-specifically degrades proteins and nucleic acids and promotes lipid peroxidation. Membrane stability index is reduced leading to increased electrolyte leakage. Lipid-protein interactions and activities of membrane ATPases are severely hampered (Das and Roychoudhury 2014). In order to sequester ROS and detoxify their effects, plants increase the activities of antioxidative enzymes like superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), *etc.* (Paul *et al.* 2017). High activities of SOD, POX and CAT have been observed in

Raphanus sativus, Coriandrum sativum, Brassica juncea, and *Spinacea oleracea* exposed to F stress (Singh *et al.* 2013, Gadi *et al.* 2016). Mondal (2017) reported high CAT activity in *indica* rice irrigated with F contaminated water. The activities of CAT and glutathione peroxidase (GPX) prominently decrease with increased F stress in *Zea mays*, thus showing the F susceptibility of this genotype (Mutahir *et al.* 2016). The accumulation of non-enzymatic antioxidants like ascorbic acid (AsA) and phenolics has also been observed in several plant species after F exposures (Table 1 Suppl.). The increase in the AsA content signifies higher efficiency of the AsA-glutathione cycle, the redox regulator in plant cells (Das and Roychoudhury 2014). Datta *et al.* (2012) reported higher activities of POX and AsA oxidase in *Cicer arietinum* plants grown under F stress in comparison to the controls. Studies covering the expression pattern of genes encoding antioxidants have not yet been reported.

F accumulation in edible plant parts

Bioaccumulation of F in the grains of staple crops like cereals and legumes and in the edible parts of vegetables is a direct threat to the entire food chain (Mondal and Gupta 2015). Popular edible plants like *Cichorium* spp., *Brassica oleracea*, *Spinacea oleracea*, *Apium graveolens*, *etc.*, accumulate high amount of F (Hong *et al.* 2016). Such accumulation occurs after efficient root-to-shoot translocation from the soil or direct absorption from the atmosphere (Ambuvel *et al.* 2015). Gupta *et al.* (2009) reported an alarming accumulation of 5 000 and 4 000 mg(F) kg^{-1}(d.m.) in the *indica* rice seedlings irrigated with water contaminated by 20 and 30 mg dm^{-3} NaF, respectively, for 15 d. The straw of such plants is unsuitable for animal feed and it is an indicator of high F accumulation also in the grains (Gupta *et al.* 2009).

Mondal (2017) demonstrated significant F accumulation in *indica* rice cultivars with maximum translocation to shoots under exposure to 5 mg dm^{-3} NaF. Ambuvel *et al.* (2015) found the extent of F accumulation to be higher than the WHO safe limit (1.5 mg kg^{-1}) in *indica* rice and black gram seedlings irrigated with groundwater in Tamil Nadu, India. The leaves of *Camellia sinensis* accumulated 214 mg(F) kg^{-1}(d.m.) when grown in F contaminated soils (Baunthiyal and Ranghar 2013).

Thus, it is advisable to prohibit the use of F contaminated water for irrigation purposes. However, in the present scenario, where large proportions of the soil, groundwater, and surface water are already affected with F contamination, new strategies for deconta-mination should be evolved.

Phytoremediation

Phytoremediation can be a cheap and environment friendly strategy for decontaminating F infested soils and waters *via* the use of green plants which have high translocation efficiency, bioconcentration factor, enrichment factor and tolerance to F (Lorestani *et al.* 2011). Such plants regarded as F hyper-accumulators can be identified by screening trees, shrubs, and aquatic plants naturally thriving in the F infested areas. The selected species can accumulate appreciable amounts of F without manifesting phytotoxic effects and they maintain high biomass (Yoon *et al.* 2006).

Table 2 Suppl. represents the list of potential plant species capable of acting as F phytoremediators. The *Prosopis juliflora* belonging to *Fabaceae* is a popular choice for cleaning contaminated soils and groundwater. The tree has an extensive and deep root system and a very high root-to-shoot translocation (Saini *et al.* 2012). F tolerant plants have been screened among the vegetation thriving around phosphate fertilizer factories due to the positive correlation between phosphate and F content in the soil (Mezghani *et al.* 2005). Whereas F sensitive apricot leaves exhibited necrotic patches at 65 mg(F) kg^{-1}(d.m.), olive trees could sustain up to 300 mg(F) kg^{-1}(d.m.) without exhibiting any injurious symptom (Mezghani *et al.* 2005). In an interesting study, the arsenic contaminated groundwater contains also unusually high content of F (Zhao *et al.* 2015). It was shown that 210 and 316 μM F accelerate As (III) and As (V) uptake by *Pteris vittata* in hydroponic cultures (Zhao *et al.* 2015). Thus, *P. vittata* may be a suitable candidate for cleaning up the groundwater contaminated with both As and F. Boukhris *et al.* (2015) identified three perennials *Rhanterium suaveolens*, *Atractylis serratuloides*, and *Erodium glaucophyllum*, which are *in situ* phytoremediators on arid F polluted soils. Three semi-arid species, *Acacia tortilis*, *Cassia fistula*, and *Prosopis juliflora* were grown in 10, 20, and 50 mg(F) kg^{-1}(d.m.) in *Soilrite* for one month and no changes in Chl fluorescence parameters were observed (Baunthiyal and Sharma 2013). However, *C. fistula* plants exhibited F sensitivity in the later growth stages. The other species were F-tolerant.

Some aquatic algae and higher plants have evolved F tolerance by increasing the endogenous Ca^{2+} content in order to sequester the free F ions (Table 2). Thus, F remains inactivated as CaF_2 in the roots and not translocated *via* the vascular tissues. Other deactivation mechanisms could be reprogramming of the cellular machinery and shift towards F-insensitive metabolic pathways (Santos-Diaz and Zamora-Pedraza 2010). Exhaustive metabolomic investigations are necessary to establish such possibilities. Karmakar *et al.* (2016)

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identified three aquatic macrophytes (*Pistia stratiotes*, *Eichhornia crassipe,s* and *Spirodela polyrhiza*) capable of removing F with an efficiency of 19.87, 12.71, and 19.23 %, respectively. Study of their physiological parameters like Chl and Car content, and CAT and POX activities showed that the plants were marginally affected during F stress and hence could be used as potential phytoremediators (Karmakar *et al.* 2016).

Intricate studies of characteristic F-induced injuries in sensitive plants can help in the environmental detection of F. These plants can be regarded as the 'bioindicators of

F pollution'. Rodriguez *et al.* (2012) monitored the F induced visible effects on the leaves of *Eucalyptus rostrata*, *Populus hybridus*, and the needles of *Pinus radiata* trees growing around aluminium smelters. The tissues accumulated 6 - 3 652 mg(F) kg^{-1}(d.m.) with the maximum accumulation in *E. rostrata*. Washing of the leaves and needles removed the external F, which reduced total F content by 24, 39, and 51 % in *E. rostrata*, *P. hybridus*, and *P. radiata*, respectively (Rodriguez *et al.* 2012).

Conclusion and future perspectives

In spite of being one of the most abundant elements and representing about 0.3 g kg^{-1} of the earth's crust, the phytotoxic effects of F have been insufficiently studied in plants. The elevated content of Fs are detected in silt clay loam soils and soil treated with phosphate fertilizers. The bioavailability of F depends on soil properties like pH, sorption capacity, and permeability. Plants can be exposed to edaphic (soil) or atmospheric (in the form of HF) F stresses. Atmospheric F is absorbed mostly *via* the stomata. The uptake of soil Fs occurs *via* the roots and the absorbed F ions are then translocated to the stems, leaves, and storage organs *via* an apoplastic route. The exact localization of the accumulated F within the cell remains unclear. F phytotoxicity is primarily manifested by leaf tip burns, leaf scorch, and chlorosis. Excess F accumulation in sensitive plants inhibits vital processes like photosynthesis, N metabolism, signalling, nucleic acid synthesis, *etc*. and stimulate ROS production. Increased accumulation of ROS damages membranes and enzyme structures, but plants also accumulate non-enzymatic antioxidants like AsA and polyphenols and

increase the activity of enzymatic antioxidants like SOD, CAT, and POX to scavenge the toxic ROS. Expression profiles of genes encoding antioxidants should be studied in future.

The accumulation of F in edible plant parts is a serious threat to the animals and humans. In order to control such biohazards, F hyperaccumulators and tolerant species can be screened for possible phyto-remediation. These species might contain very high content of antioxidants and efficient compartmenta-lization or effective export of F ions.

Phytoremediation is an easy and cost-effective approach to remove F from contaminated soils and water sources. However, the strategy is time consuming and sometimes partly efficient (Roy *et al.* 2015). In situations where the cultivated field is contaminated with F, it would be economically challenging firstly clean the soil by planting F hyperaccumulators and after that cultivate crops or vegetables. Hence, it is important to genetically modify the crop plant to produce F-tolerant genotypes with low F bioaccumulation.

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