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Fluorides and Its Effects on Plant Metabolism

Baunthiyal, M.[*], Bhatt, A. and Ranghar, S.

Department of Biotechnology, GB Pant Engineering College, Pauri Garhwal, Uttarakhand, India.

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Abstract Fluoride is one of the most important phytotoxic air pollutants. It is toxicity to terrestrial plants that has been reviewed and clearly demonstrated in laboratory, greenhouse and controlled field plot experiments.

Keywords: fluoride, phytotoxic, pollution

Introduction

Fluoride injuries to plants may be acute or chronic. Acute F injury is caused by short-term exposures to high concentrations of atmospheric F. Injury symptoms similar to those caused by atmospheric fluorides may also be due to other agents, such as insects, disease, nutritional disorder and adverse weather. F injury to vegetation commonly results from gradual accumulation of F in the plant tissue over a period of time (Davison, 1984). Therefore the duration of exposure as well as the atmospheric concentration is important in determining the severity of injury. Fluoride (F) is one of the most important phytotoxic air pollutants (Weinstein, 1977; Doley, 1986, 1989; McCune *et al*., 1991).

Uptake, Translocation and Accumulation

Accumulation by terrestrial plants

Terrestrial plants may accumulate inorganic fluorides following airborne deposition and uptake from the soil (Davison, 1983). When F is present both as an air and soil pollutant, the uptake from air (through stomata) is far more significant than from soil (Groth, 1975). However analysis show that plant roots can contain very high F concentrations (1,000 to 6,000 $\mu g.g^{-1}$ dw) when F enters from the soil, whereas the concentration is very low (about 10 $\mu g.g^{-1}$dw) in roots when F enters *via* the leaves (Pitman, 1982). Sloof *et al.* (1989) reported that the main route of uptake of F by plants is from aerial deposition

[*] **Corresponding author:** Baunthiyal, M.; **Email:** mamtabaunthiyal@yahoo.co.in

on the plant surface. Gaseous F enters the leaf through the stomata and dissolves in the water permeating the cell walls. The natural flow of water in a leaf is towards the sites of greatest evaporation, which are the margins and tip. Carried by water, F concentrates in the margins and tip, so these areas generally are the first to show visible injury. This concentration mechanism is one reason why F is so toxic to plants but there is an important corollary; most of the leaf may have very little F present and may function normally in terms of assimilation. The ultimate factor determining the severity of injury is the amount of fluoride reaching active sites and remaining there in toxic amounts.

Uptake of F by roots is a passive, diffusion process (Venkateswarlu *et al*., 1965; Cooke *et al*., 1978; Garrec and Letoureneur, 1983) in which most of it remains exchangeable and readily extractable from the root by mild washing procedures. Probably, most of the F in roots is in the apoplast (cell walls and intercellular spaces) and correspondingly little passes through the plasmalemma or tonoplast. The endodermis acts as an effective barrier to the vascular tissue so transport to the shoot is limited. F reaches the vascular system by a non-selective route that bypasses the endodermis (Pitman, 1982; Davison *et al*., 1985) and the concentration in individual leaves may be function of concentration in the rooting medium and of water flow.

Considerable experimental work has been done on plants regarding their capacity to accumulate F. Plant uptake of F from solution culture is dependent on plant species and positively related to the ionic strength of the growth solution. Once a threshold F ion activity in nutrient solution was reached, F concentrations in plants increased rapidly (Stevens *et al*., 1998a). A number of factors influence the action of fluorides on vegetation and the uptake and accumulation of F by plants (McCune *et al*., 1965; Davison *et al*., 1985). Soil type, Ca and P content and soil reaction (pH) seem to be the predominant controlling factors (Sheldrake *et al*., 1978). For example, Conover and Poole (1971) found that calcite clay prevented the uptake of water-soluble F, whereas sphagnum peat did not have the same capability. The uptake of F in plants did not increase even after substantial additions of F compounds to well-limed soils as F combined with calcium to form a very insoluble CaF_2 and thus was no longer available in soil solution for plant uptake. At acidic pH (below pH 5.5), F becomes more phytoavailable through complexation with soluble aluminium fluoride species, which are themselves taken up by plants or increase the potential for the F ion to be taken up by the plant (Stevens *et al*., 1997). It was suggested that the wide differences often found between plant species and varieties with respect to degree of F accumulation may be explained by means of uptake, translocation and distribution of F. Plants absorb F in the unidirectional distal movement, which eventually results in its accumulation in

their leaves, roots and fruits. Highest F concentration was found in roots and lowest in fruits (Singh *et al*., 1995). An experimental data was presented which demonstrated both basipetal and acropetal translocation of F in corn leaves (Bligny *et al*., 1978).

F uptake from soil by plants is generally low (except for accumulators) unless it has been added suddenly, such as following amendment with sludge or phosphate fertilizer. The amount of F taken up from soil by sunflower seedlings (*Helianthus annuus*) was proportional to the F concentration of the solution added to the soil (10–100 $\mu g.ml^{-1}$). Highest concentrations were found in the roots, with reduced concentrations in successive leaf ranks (Cooke *et al*., 1978).

Kornberger *et al*. (1978) performed a large number of analyses to determine the F concentration in various parts of corn plants at specific stages of growth. There were extreme variations in F levels, which were related to differences in exposure to emission, translocation phenomenon within the plant and effect of dilution by intensive biomass production. Thus F concentration of husks showed a continuous decrease from the outer to inner surfaces. When comparing exposed and covered portions of styles, F concentration were found 18 times higher in exposed tips. A similar descending trend occurred in comparing the blade of leaves, the sheath and the stalk. The extremely high amount of F was found in tassel of corn and was possibly because of its glutinous surface, making it more susceptible to particulate F contamination. This may also be true for exposed parts of style.

The uptake of F by ryegrass (*Lolium multiflorum*) grown in soil amended with a F-rich sludge increased with increasing F application (84-672 $kg.ha^{-1}$) (Davis, 1980). The F concentration was less in shoots compared to the leaves. The F concentrations in bark and shoots of trees of Rowan *(Sorbus aucuparia)* were mostly low compared with leaves, but the bark of *Betula pendula* and *Betula pubescens* had very high concentrations (Hogskolevein, 1997). Similarly, concentrations of F were high in roots and low in shoots in tomatoes (*Lycopersicon esculentum*) and oats (*Avena sativa*) grown on F-enriched media (Stevens *et al*., 2000).

Increasing bioavailable Ca results in increased root F content but lower leaf 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 is least preferred organ of F accumulation. Studies by Singh *et al*. (1995) on the F uptake in irrigation water by *Abelmoschus esculentus* (ladyfinger), showed that the roots accumulated most of the F supplied through the irrigation water, while the fruit accumulated least. In soil

culture, the accumulation of F in the different plant parts followed the trend as root > leaves > fruit > shoot.

Reynolds and Laurence (1990) found that exposure of kidney bean (*Phaseolus vulgaris*) plants to intermittent high levels of atmospheric HF (3 µg $F.m^{-3}$ for 5 consecutive days or 5 µg $F.m^{-3}$ for 3 consecutive days) caused higher accumulation in leaves than did exposure to a continuous dose at a lower level (1 µg $F.m^{-3}$ for 15 consecutive days), even though the plants received the same total dose. They also noted that the rate of uptake from the air over a 15-day period, particularly for the highest dose (5 µg $F.m^{-3}$), was most significant at first exposure and declined during subsequent exposures.

Most species take up little F from the soil but some are exceptional, accumulating several hundred $mg.g^{-1}$ from soils that have concentration with in normal range. Members of tea family (Theacea) are best-known examples (Table 1). Tea plant (*Camellia sinensis*) accumulates large amount of F in mature leaves from soil of normal F availabilities (Ruan *et al.*, 2003), but the properties of F absorption by this plant species are not well understood. Analysis of the successive leaves of cultivars of tea plant showed that there is a stronger correlation between F and Al, than between F and other elements with which it might be expected to be associated (e.g. calcium; Weinstein and Alscher-Herman, 1982). Physiological studies have shown that if the two elements are present in the rooting medium, then accumulation and transport of both is enhanced. The F taken up by tea plants was largely and readily transported, in particular to the leaves. The F in leaves increased linearly with its concentrations in the uptake solution or in the soil, whereas those in root and stem were only marginally influenced. Including Al in solution or adding Al to the soil apparently increased the uptake and translocation of F to the leaves. The concentrations of F in the leaves were significantly increased by 19.1% or 37.7% when 18.5 µmol.l^{-1} Al or 74.1 µmol.l^{-1} Al respectively, was included in the uptake solution, compared with the control without Al during an uptake period of 22 hrs. Similarly, Al application (100 $mg.kg^{-1}$) to soil led to significantly higher F concentrations in mature leaves and new shoots (one bud with three leaves). By contrast, the concentrations of Al in leaves in solution and soil experiments were not affected by F and Al treatments. Nevertheless, higher Al concentrations after Al and F additions were observed in the new shoots in soil experiments. As Theacea are also known to be aluminum accumulators, Davison (1982) speculated that there might be a link between the elements and analyzed a range of species that were known to be aluminium but not F, accumulators. Of the five families investigated, all had species that showed accumulation of F upto several hundred $\mu g.g^{-1}$ (Table 1). Stevens *et al.* (1997) showed a correlation between the activity of $AlF^{2+}$and AlF_2^{+} and the

concentrations of Al and F in oat and tomato plants. They suggested that AlF^{2+} and AlF_2^+ are taken up by the plants and translocated to the shoots. The mechanism for uptake of AlF_n complexes from solution is not clear, but several possible pathways can be suggested. The first possibility, invoked for higher plants, would be the uptake and translocation of intact metal-ligand complexes through the apoplast (Watanabe *et al*., 2001). The second route of entry would involve the formation of mixed ligand (fluoro-Al-membrane) complexes at transport sites present at plant surface, followed by transport of the intact AlF_n complex across the plasmalemma (Campbell, 1995). According to Miller *et al*., (1985) the first major resistance barrier to cellular F accumulation imposed by the plant is the cell wall. The high F concentration within the cell wall is in accordance with the known high calcium concentrations of that fraction. The cell wall accumulates F until all the Ca^{+2} present in cell wall is bound. This phenomenon might account for the difference in the tolerance of different plant species to threshold levels of accumulated fluoride as the cell wall Ca^{+2} would be acting as a buffer against cellular F accumulation. Even slight elevation in the level of F above the buffering capacity of the cell wall will very rapidly result in high level of organelle accumulation. The mechanism as to how F enters the cell is unknown because the uptake of F into cells is rapid and occurs in minutes. The uptake of F is enhanced by chloride deficiency and F like chloride is halide, so it is reasonable to speculate that cellular uptake of F is mediated through the chloride channel.

F toxicity is a pH-related phenomenon. Toxic action starts by damage to the transport channels associated with the cytoplasmic membrane. Being a very strong H-bonding agent, F is able to couple with virtually most of the cell constituents. Driven by transmembrane pH gradients and concentration difference, partitioning of accumulated F to sub-cellular organelles continues till organelle function is lost (Armstrong and Singer, 1980).

Table 1. F concentration ($\mu g.g^{-1}$) in species screened for accumulation (Data from Davison, 1982)

Species	**F Accumulation ($\mu g.g^{-1}$)**
Theaceae	
Camellia sinensis, tea	214
Stuartia pseudocamellia	50
Stuartia pteropetiolata	452
Ternstroemia gymnanthera	58
Eurya emarginata	1655
Diapensiaceae	
Diapensia lapponica	361
Pyxidanthera barbulata	280
Schizocodon soldanelloides	37
Galax urceolata	394
Shortia uniflora	136
Melastomataceae	
Tibouchina organensis	65
Tibouchina paratropica	41
Tibouchina urvilleana	10
Rubiaceae	
Psychotria brasiliensis	60
Psychotria jasminiflora	400
Psychotria mucronata	33
Psychotria hillebrandii	43
Rudgea leucocephla	546
Cyatheaceae	
Cyathea australis	396
Cyathea malzinei	119
Symplocaceae	
Symplocos paniculata	56

Miller *et al*. (1985) proposed a steady-state model for F transport across the membranes and its sub-cellular partitioning. The basis for this model resides in the weak acid characteristics of HF (pKa 3.45), which would be in 2 forms, HF and F^-, and directly dependent on the pH of the immediate microenvironment. This can be calculated mathematically by the Henderson-Hasselbalch equation:

$$pH = pKa + \log(F^-)/(HF)$$

The two F forms differ by 6 magnitudes in their ability to pass through lipid membranes. Thus concentration differences of F between the apoplast (outside cell wall) and the cytoplasm, mitochondria and chloroplast would be great. If 1.9 ppm F were in the apoplast (pH 5.8) then 47, 298 and 190 ppm

would be found in the cytoplasm (pH 7.2), chloroplast (pH 8.0) and mitochondria (pH 7.8) respectively (Fig. 2.3). Such a model indicates that low F levels in the apoplast can be concentrated to high levels in organelles, which could induce physiological damage.

Visible symptoms of F injury

Chlorosis and necrosis have long been recognized as the first visible symptoms of F injury to plants (McNulty and Newman, 1957). These symptoms occur when extraneous soluble F compounds are introduced into the environment of either the roots or the leaves. In some cases, the lethal threshold concentration of tissue F is reached suddenly, as the cells appear quite normal upto the time of visible injury.

In most monocotyledonous and dicotyledonous plants, the initial symptom was the development of chlorosis at the tips and margins of elongating leaves, which later extended downwards along the margins and inwards toward the midrib. This chlorosis became more intense and extensive with prolonged exposure until the midrib and some veins appeared as a green arborescent pattern on a chlorotic background. Continued exposure may lead to the tip becoming necrotic and falling off, leaving the leaf notched (plate 2.1) (McNulty and Newman, 1961; Weinstein and McCune, 1971; Weinstein and Davison, 2003).

Exposure to a high concentration of F caused necrosis of part or even whole of the leaf. The initial stages vary with species and both the speed of development of the symptoms and their appearance depend on the weather. Hitchcock *et al*. (1962) reported necrotic leaf tips in gladiolus (*Gladiolus* sp.) cultivars exposed to 0.17 µg $F.m^{-3}$ for 9 days.

Wolting (1975) observed leaf necrosis on freesia (*Freesia* sp.) cultivars during continuous fumigation at 0.5 µg $F.m^{-3}$ for 5 months and during intermittent fumigation with 0.3 µg $F.m^{-3}$ (6 h/day, 3–4 times/week) for 18 weeks.

Coniferous trees have been identified as sensitive plant species for F. In field exposure chambers, significant dose–response relationships were observed between HF exposure and development of needle necrosis in 2-year-old black spruce (*Picea mariana*) and 3-year-old white spruce (*P. glauca*) (McCune *et al*., 1991). Four test chambers, one of which served as a control, were used for different HF exposure regimes for each coniferous species. Measurements of tissue necrosis were recorded after 10 days following HF exposure for 78 h with black spruce and 20 days following HF exposure for 50 h with white spruce. Lowest-observed-effect concentrations (LOECs) for necrosis were 4.4 and 13.2 µg $F.m^{-3}$ for black spruce and white spruce, respectively.

Murray (1984) exposed grapevines (*Vitis vinifera*) to HF concentrations of 0.07 (control), 0.17 and 0.27 µg $F.m^{-3}$ for 189 days. Foliar necrosis was first observed on vines and both chlorophyll a and total chlorophyll were significantly reduced by F exposure. F had no significant effect on bunch weight, number of bunches, grape yield, grape water or potential alcohol content, leaf chlorophyll b or leaf protein concentration.

In some species the dead, necrotic areas were pale white to tan, in others they were brown and they may be black (e.g. in *Populus* sp.) or have reddish tinges. Characteristically, there was a dark brown margin along the basal part of the necrotic area. This line of demarcation was very useful in identifying multiple exposures. The necrotic area was sharply delineated from the healthy portion of the leaf blade by a narrow band of chlorotic tissue sometimes streaked with red as in some varieties of *Sorghum*. Dead, dry pieces of leaf may become brittle and fall off, giving the leaf a tattered appearance. This was common in Chinese apricot and Italian prune and many *Populus* varieties.

When very young leaves were injured in this way the resulting leaf was only a fraction of the normal size and completely mis-shaped (Weinstein and Davison, 2003).

Pack (1971) found no effect of HF on the progeny of bean plants (no species stated) grown at 0.58 µg $F.m^{-3}$. However, the primary leaves of some F_1 progeny of plants grown at $\geq$2.1 µg $F.m^{-3}$ were severely stunted and distorted.

MacLean *et al*. (1984a) exposed wheat (*Triticum aestivum*) and two sorghum (*Sorghum* sp.) hybrids to HF at concentrations ranging from 1.6 to 3.3 $\mu g.m^{-3}$ for three successive 3-day periods. Anthesis (the maturing of the stamens) was the most sensitive stage, and this occurred during the first exposure period in wheat and the third exposure in sorghum. HF-induced foliar damage did not occur in wheat; however, both sorghum hybrids developed foliar damage in proportion to the exposure concentration.

Airborne F can also affect plant disease development, although the type and magnitude of the effects are dependent on the specific plant–pathogen combination (Laurence, 1983). Van Bruggen and Reynolds (1988) found that exposure of soybean (*Glycine max*) plants to HF (2 µg $F.m^{-3}$) in a controlled chamber led to significantly larger hypocotyl lesions after inoculation with either *Rhizoctonia solani* or *Phytophthora megasperma*. Plants were exposed to HF for 1 week before and after the infestation. However, Laurence and Reynolds (1984) found no significant effect of HF (1 or 3 µg $F.m^{-3}$ for 5 days) on lesion characteristics of the bacterium *Xanthomonas campestris* on the leaves of red kidney bean (*Phaseolus vulgaris*) plants. Similar findings were reported by Reynolds and Laurence (1990) during both continuous (1, 3 or 5 µg $F.m^{-3}$ for 15, 5 or 3 days, respectively) and intermittent (3 or 5 µg $F.m^{-3}$ for 15

days) exposures. Signs of inorganic F phytotoxicity (fluorosis), such as chlorosis, necrosis and decreased growth rates, were most likely to occur in the young, expanding tissues of broadleaf plants and elongating needles of conifers (Pushnik and Miller, 1990).

Generally, leaves are most sensitive when they are young and still expanding. Once fully developed they may be many times more resistant. Therefore symptoms are more often seen in young, expanding leaves (Weinstein and Halscher-Herman, 1982). In young, developing leaves of broad-leaved species, and occasionally in petals, the translocation of F to the margins and tips leads to a distorted shape. This occurs because cells in the mid parts of the leaf have low F and expand normally but those on the margins are slower-growing so the leaf buckles and distorts, becoming cupped and concave or convoluted like a savoy cabbage (Weinstein and Davison, 2003).

Where fumigation is periodic, only those leaves that are at the sensitive stage of development will develop injury. The rate at which symptoms appear depends on the weather. There can be a considerable lag between the time of exposure to the F and the development of the symptoms. Klumpp et al. (1996) found a highly significant linear regression between leaf damage and F accumulation in *Gladiolus* plants; the plants developed typical F-induced leaf lesions.

There is little information about the effects of F on fruits. Peach (*Prunus persica*) showed an unusual serious physiological disorder induced by F called "suture red spot" or "soft suture" of the fruit. It was characterized by premature ripening of the flesh on one or both sides of the suture toward the stylar (blossom) end of the fruit. The ripening of this tissue considerably preceded that of the normal fruit and was often accompanied by splitting of the flesh along the suture. At harvest, the affected areas were soft and often decomposing (MacLean *et al*., 1984b).

However, there is little or no relationship between visible injury and either growth or longevity. A plant that is visibly injured is not necessarily dying and there have been some cases of spectacular recovery of trees after severe injury. Many that show a significant degree of injury (such as *Populus* sps.) continue to grow at normal rates. Conversely, just because a plant does not show visible injury it does not mean that there is no effect of F on assimilation or growth (Weinstein and Davison, 2003).

Histologically F causes rather distinctive injury to cells and tissues, which can be identified following proper sectioning (Treshow, 1971). The microscopic symptoms of pine needle injury consisted of enlargement of phloem and xylem parenchyma, and enlargement and distortion of transfusion parenchyma cells. Cells were injured only in narrow band extending less than a

millimeter beyond the necrotic area. The leaves of F-treated and control plants were sectioned and examined microscopically. As chlorosis developed, there was a simultaneous disintegration of the chloroplasts. This occurred prior to any apparent disruption of the cell. The breakdown of chloroplasts and simultaneous mixing of their contents with the cytoplasm could explain the decrease of all chloroplast pigments occurring simultaneously and to the same degree (McNulty and Newman, 1961).

Zwiazek and Shay (1987) grew jack pine (*Pinus banksiana*) seedlings in sand culture at 3 or 15 mg $F.kg^{-1}$ dry weight. Wilting was the first sign of F injury and occurred in approximately 50% of plants after 25–26 h at 15 mg $F.kg^{-1}$ and 2–6 h later in only 7% of plants exposed to 3 mg $F.kg^{-1}$. F-induced injuries to mesophyll and guard cells were similar to those caused by drought and included the appearance of lipid material in the cytoplasm during early stages of injury, suggesting cell membrane damage. Ultrastructural changes in leaves due to NaF treatments confirmed the severe collapse of mesophyll cells, their plasma membrane having become detached from the cell wall and in some cases, broken (Fornasiero, 2001).

There has always been some difficulty in distinguishing symptoms of F injury from those caused by unrelated environmental stresses or parasitic diseases. The distinction must be made before the significance of a F pollution situation can be assessed (Treshow, 1971). The rate at which symptoms appear depends on many environmental factors, such as (i) type and concentration of pollutant(s), (ii) distances from source, (iii) length of exposure and (iv) metereological conditions (Fornasiero, 2003).

Effects on growth and development

1000 to 1500 ppm F added to soil in one experiment reduced the yield of winter wheat by up to 65% and 400 ppm reduced growth of T*radescantia* (Garber, 1968) a flowering plant by 28 to 34%. Hadjuk in 1969 reported a strong correlation between inhibition of pea seedling growth and increased F content of the soil. Davis and Barnes (1973) found that an added soil F concentration of 380 mg kg^{-1} significantly reduced the growth of loblolly pine (*Pinus taeda*) and red maple (*Acer rubrum*) seedlings.

Cooke (1976) studied the effect of F (200 ppm) on common sunflower (*Helianthus annus*) seeds grown in sand culture. No effect on total dry weight was observed; however, there were significant reductions in leaf growth. MacLean et al. (1977) observed a significant reduction (25%) in the fresh mass of pods from bean plants (*Phaseolus vulgaris*) exposed to 0.6 µg $F.m^{-3}$ for 43 days (emergence to harvest) in open-top field chambers. There was no effect of HF exposure (0.6 µg $F.m^{-3}$) on growth or fruiting in tomato (*Lycopersicon*

esculentum) plants. MacLean and Schneider (1981) found a significant reduction (20%) in the mean dry mass of wheat plants (*Triticum aestivum*) exposed to 0.9 µg $F.m^{-3}$ for 4 days.

Doley (1986) fumigated three varieties of grapevine with HF for four successive growing seasons (the duration of exposure varied from 54 to 159 days for each season). Leaf size during the first growth of the season was not affected by F concentrations upto 1.5 µg $F.m^{-3}$. However, leaf size was significantly reduced during the middle and latter portion of the fourth season of fumigation in two of the varieties at 0.64 µg $F.m^{-3}$.

Several species of *Eucalyptus* have shown sensitivity to F. Murray and Wilson (1988b) exposed *Eucalyptus tereticornis* to HF (0.38 µg $F.m^{-3}$) for 90 days in open-top chambers. F significantly reduced leaf surface area and weight in mature and immature leaves. The same significant effects on immature leaves were noted for marri (*Eucalyptus calophylla*), tuart (*Eucalyptus gomphocephala*) and jarrah (*Eucalyptus marginata*) at 0.39 µg $F.m^{-3}$ for 120 days. However, in mature leaves, leaf surface area and weight were reduced in tuart, and surface area was reduced in marri. In jarrah, these two end-points were unaffected (Murray and Wilson, 1988 a).

Belandria *et al*. (1989) studied the effect of NaF on the germination of lichen ascospores. They found that only 20% of the *Xanthoria parietina* spores were able to germinate in the presence of 19 ppm F. Several studies on F have been carried out in culture media. Stevens *et al*. (1998a,b) grew tomato (*Lycopersicon esculentum*) and oat (*Avena sativa*) plants in nutrient solutions (12–13 days) at NaF concentrations ranging from 1 to 128 ppm. F ion concentrations greater than 28 ppm caused significant decrease in the dry weights of tomato shoots and roots, whereas oat plants were unaffected at all F exposure concentrations. They also found that dry weights of tomato and oat shoots and roots were significantly decreased as the pH of the solution culture decreased below 4.3 at HF concentrations of 1.4 ppm F and 2.6 ppm F for tomatos and oats, respectively. F concentrations in tomato and oat shoots, which corresponded with significant reductions in plant dry weights, were 228 and 125 $mg.kg^{-1}$, respectively.

Elrashidi *et al*. (1998) found that an application of 100 ppm F significantly reduced dry matter yield of barley (*Hordeum vulgare*) grown on both acid (pH 4.75) and neutral (pH 6.6) soils for 40 days; however, plants grown on alkaline soil (pH 7.5) were unaffected at 1000 ppm F.

Effects of different NaF concentrations on the growth and certain metabolic parameters of almond seedlings *(Amygdalus communi*s) were studied under strictly controlled growth conditions in nutrient solutions containing increasing NaF concentrations ranging from 0 to 10 mM NaF. After 14 days

dry matter was significantly reduced in the root system, which accumulated large amounts of F. The chlorophyll, Ca and Mg content of the leaves showed a significant decrease, and the starch and sugar content in leaves was also reduced, especially at the higher F concentrations. However, in F-exposed almond seedlings, root Mg, Ca, and Fe contents seem to be unaffected except for Mn, which showed a major decrease at 2.5 mM NaF. Overall, the nutritional status of the leaves appeared to be affected more than that of roots (Elloumi *et al*., 2005).

The influence of 0 to 30 µM NaF on the germination, seedling growth and total biomass of the cluster bean *(Cyamopsis tetragonoloba*) was studied under strictly controlled conditions. At the end of 15 days of treatment, significant reductions in percent seed germination, root and shoot length and total biomass were observed at increasing F concentration. At 30 µM NaF concentration, 100% mortality of the seeds occurred (Sabal *et al*., 2006).

Bhargava and Bhardwaj (2010) studied the effects of 4, 8, 12, 16 and 20 mg/L sodium fluoride (NaF) on *Triticum aestivum* var. Raj. 4083 seeds and seedling growth. After 7 days of treatment with control, 100% germination occurred, but at 20 mg NaF/L, germination was reduced to 88%. Physiological parameters, viz., root length, shoot length and dry weight decreased with increasing NaF concentration. At 20mg NaF/L, the average root length, shoot length and dry weight were reduced by 36.6%, 24% and 20.54% respectively. At 20 mg NaF/L, Vigor index was reduced by 37.20% compared to control. The chlorophyll content of the leaves was also reduced monotonically. At 20mg NaF/L, it was 0.074 mg/g which was 27.45% lesser compared to control. Ascorbic acid content initially decreased and then increased with increasing concentration of NaF (20mg/L).

Gadi (2012) studied the effect of sodium fluoride (0.1, 0.25, 0.50, 0.75 and 1 mM NaF) on germination behaviour, membrane stability and some biochemical parameters in in-vitro grown seedlings of Vigna radiata L. After 7 days of treatment germination percentage, root length, shoot length, vigour index, percentage ofchlorophyll stability index (CSI), membrane stability index (MSI) and soluble protein content were decreased in seedlings under fluoride stress. Sodium fluoride (NaF) treatment resulted in a significant enhancement of osmolytes such as proline and total soluble sugars content. The results of there experiment indicated that NaF disturbed the seed germination, seedling growth and membrane stability whereas, increased proline and carbohydrates in Vigna seedlings.

Datta *et al*. (2011) studied the influence of 0, 0.1 mM, 0.5 mM, 1.0 mM, 4.0 mM, 8.0 mM fluoride (F) concentration on seed germination, seedling growth of gram seeds (cv. Anuradha) under laboratory condition. At the end of

15 days of treatment, significant reduction in root length, shoot length, dry weight, fresh weight, % of germination, protein content, catalase activity, tolerance index, vigour index, germination rate, germination relative index, mean daily germination were observed at increasing fluoride concentration. Total soluble sugar content, proline content, peroxidase activity, ascorbic acid oxidase activity, % DFC, % phytotoxicity of root and shoot increased along with gradual increment of F concentration. 4.0 mM F concentration was found to be most sensitive for gram seeds. At 8.0 mM F concentration germination occurred but plants were totally dried after completion of treatment period.

Effects on Biochemical processes

Effects on proteins, organic acids and sugars

Yang and Miller (1963) studied the metabolic processes associated with free sugars, organic acids and amino acids in higher plants subjected to F fumigation. F-fumigated leaves contained more reducing sugars and less sucrose than the normal leaves. This result suggested inhibition of sucrose synthesis by F. Necrotic leaves contained increased organic acids, which were mostly attributable to malic acid, malonic acid and citric acid. The greater increase in malic acid relative to that of citric acid was the reverse of results observed in chlorotic tissue. Necrotic leaves also contained enhanced amounts of free amino acids. The greatest increase occurred in the concentration of asparagine and might be related to the increased respiratory rate of necrotic leaves. Pipecolic acid accumulated in large quantities in necrotic tissue and was not detected in normal leaves. The accumulation of organic acids and amino acids in leaves during F fumigation was evidenced by a lowered respiratory quotient. Dark CO_2 fixation and phosphoenolpyruvate-carboxylase activity were studied in F-necrotic and control soya-bean leaves. Results suggested that the accumulation of organic acids and amino acids in necrotic leaves resulted from an increased rate of dark CO_2 fixation. In soyabean (*Glycine max*) also, F is known to increase foliar levels of free amino acids (Jagar and Grill, 1975).

These observations have been associated with visible symptoms after HF fumigation. In a study by Hautala and Holopainen (1995), the application of 200 ppm of F caused increase of several amino acids in barley, although visible symptoms were not detectable. It suggested the degradation of proteins under F stress.

Suppressed protein synthesis and acceleration in its degradation in plants in response to salt and metal stress has been reported by a number of workers. Several proteins are synthesized and accumulated in plant tissues under a range of stress conditions. Such proteins, referred to as stress proteins, have been noted to be induced in response to high temperature, low temperature, salinity,

drought and several other stress factors (Pareek *et al*., 1997, 1998). Wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) showed no effect of F treatment on yield when exposed to HF (0.38 $\mu g.m^{-3}$) for 90 days. There was, however, a significant increase in the grain protein concentration of F-exposed barley plants (Murray and Wilson, 1988c).

NaF inhibited both protein and DNA synthesis in cultured mammalian cells. The inhibition of DNA synthesis may be a secondary effect of the inhibition of protein synthesis, or a result of the direct inhibition of DNA polymerase (Department of Health and Human Services, 1991). While studying the effects of F on protein secretion and removal during enamel development in the rat, Den Besten (1986) found that the amount of protein present in equivalent quantities of enamel was increased further in animals given 50 ppm F in drinking water. Densitometric tracings showed that the largest amount of protein was present in enamel from animals ingesting 100 ppm F in drinking water. The increase in protein was apparent only in the lower molecular-weight proteins (below 30,000), and was not observed in the higher-molecular-weight enameling.

Effects on photosynthesis

General considerations

Photosynthesis involves reactions at five different functional levels viz. process at the pigment level, primary light reactions, thylakoid electron transport reactions, dark-enzymatic stroma reactions and slow regulatory feedback processes (Maxwell and Johnson, 2000). In principle, chlorophyll fluorescence can function as an indicator at all of these levels of the photosynthesis process as chlorophyll is the major antenna pigment, funneling the absorbed light energy onto the reaction centres, where photochemical conversion of the excitation energy takes place. Each quantum of light absorbed by a chlorophyll molecule raises an electron from the ground state to an excited state. Upon de-excitation from a chl a molecule from first excited singlet state to ground state, a small proportion (1 or 2% of the total light absorbed) of the excitation energy is dissipated as red fluorescence (Evans and Brown, 1994). The indicator function of chlorophyll fluorescence arises from the fact that fluorescence emission is complementary to alternative pathways of de-excitation, which are primarily photochemistry and heat dissipation (Mathis and Paillotin, 1981; Krause and Weis, 1991; Papageorgiou, 1996). Generally, fluorescence yield is highest when photochemistry and heat dissipation are lowest. Therefore, changes in the fluorescence yield reflect changes in photochemical efficiency and heat dissipation (Krause and Weis, 1991; Bolhàr-Nordenkampf and Öquist, 1993; Papageorgiou, 1996).

Measurement of chlorophyll fluorescence provides a rapid and non-invasive tool to detect key aspects of photosynthetic light capture, electron transport and photoinhibition (Evans and Brown, 1994; Maxwell and Johnson, 2000). The fluorescence signal is rich in information, and in plants the parameters F_v/F_m (ratio of variable to maximum chl a fluorescence in dark adapted leaves), F_v'/F_m' (ratio of variable to maximum chl a fluorescence in light adapted leaves), q_P (coefficient for photochemical quenching), q_N (coefficient for non-photochemical quenching), NPQ (non-photochemical quenching), and ΦPS II (quantum yield of electron transfer at photosystem II) are empirically verifiable indices of photosynthetic performance and acclimation status (Schreiber *et al.*, 1986; Genty *et al.*, 1989; Schreiber *et al.*, 1995a,b).

Although the total amount of fluorescence is very small, measurement is quite easy. Exposing a leaf to light of defined wavelength and measuring the amount of light re-emitted at longer wavelengths can quantify the fluorescence yield. The use of modulated measuring systems has revolutionized the application of chlorophyll fluorescence (Quick and Horton, 1984). In such systems, the light source is modulated (switched on and off at high frequency) and the detector is tuned to detect only the fluorescence excited by the measuring light. In order to gain useful information about the photosynthetic performance of a plant from measurements of chlorophyll fluorescence yield, it is necessary to be able to distinguish between photochemical and non-photochemical quenching. Modulated fluorometers specifically detect and amplify only the fluorescence excited by high intensity and short duration flash of light. The excitation intensity is constant and changes in the fluorometer signal reflect changes in fluorescence yield. Upon the application of a saturating flash (8000 $\mu mol.m^{-2}s^{-1}$ for 1 s), fluorescence raises from the ground state value (F_o) to its maximum value, F_m. In this condition, Q_A, the first electron acceptor of PS II, is fully reduced. This allows the determination of the maximum quantum efficiency of PS II primary photochemistry, given by $F_v/F_m = (F_m-F_o)/F_m$ (Genty *et al.*, 1989). In healthy leaves, this value is always close to 0.83 (Björkman and Demmig, 1987), independently of the plant species studied. A lower value indicates that a proportion of PS II reaction centers are damaged, a phenomenon called photoinhibition, often observed in plants under stress conditions (Baker *et al.*, 1994). Upon subsequent application of constant illumination, a transient rise in fluorescence yield is observed. This is due to the lag phase before carbon fixation starts. Whilst electron transport starts within milliseconds upon illumination, some carbon fixing enzymes need to be light activated. As a consequence, a substantial proportion of Q_A (the primary electron acceptor after PS II) is reduced during the first seconds of illumination

and the fluorescence yield increases. Thereafter, upon the onset of photochemical and heat dissipation processes, the fluorescence yield is quenched and reaches a steady state value (F_t) (Kautsky *et al*., 1960). Upon the application of a second saturation flash in the presence of actinic light, the maximum fluorescence obtained (F_m') is lower to that observed in the dark (F_m). Since at F_m', Q_A is fully reduced again, the difference between F_m' and F_t reflects the photochemical part of fluorescence quenching, and the difference between F_m and F_m' reflects fluorescence quenching due to heat dissipation (Maxwell and Johnson, 2000; Fracheboud and Leipner, 2003).

Thus, chlorophyll fluorescence is very useful to study the effects of environmental stresses on plants since photosynthesis is often reduced in plants experiencing adverse conditions, such as water deficit (Epron *et al*., 1992), temperature, nutrient deficiency, polluting agents, attack by pathogens. Chlorophyll fluorescence was used to investigate the effects of temperature stress on water splitting, on the efficiency of PS II reaction centers and on the light harvesting complexes of maize leaves (Leipner *et al*., 1999; Sinsawat, 1999).

Effects of F on chlorophyll a fluorescence

Chlorophyll a fluorescence was used to determine the effects of treatment with gaseous HF or aqueous solutions of NaF on the photosynthetic apparatus of spinach prior to the appearance of visible injury. Placing the petioles in 2 mM NaF for 3 h resulted in the accumulation of 240 ppm F in leaf blades. The second oldest leaves of spinach plants accumulated similar concentrations (270 ppm F) when the plants were exposed to gaseous HF for 6 days. These NaF and HF treatments did not result in visible injury nor did they affect F_o, F_m or F_v/F_m. However, the primary F-induced change observed was a more rapid quenching of fluorescence after the maximum peak (P) was reached (2 to 30 s). Quenching analysis indicated that increased photochemical quenching occurred during that time period. Electrons were rapidly moved away from Q_A, leaving a greater proportion of Q_A in an oxidized state. Concomitant with this was a slight decrease in non-photochemical quenching, suggesting that either a smaller proton gradient was maintained across the thylakoid membrane, or that slight configurational changes in the thylakoid membranes reduced their energy-dependent quenching capacity. One possible explanation could be that the thylakoid membranes may be slightly uncoupled (i.e. leaky to protons) allowing PS II to turn over at a faster rate, limiting the development of a large pH gradient. This would allow for less 'back pressure' on PS II and a reduction in energy-dependent, non-photochemical quenching (Boese *et al*., 1995).

Pukacki (2000) studied the effects of sulphur, F and heavy metals pollution on the chlorophyll fluorescence of Scots pine (*Pinus sylvestris*) needles. Scots pine trees indicated physiological adaptation to environmental pollution and this was monitored by the fluorescence signals earlier than by other methods.

Junior *et al.* (2007) studied early effect of fluoride emission injury on two tropical grasses, *Chloris gayana* and *Panicum maximum*, During an 8-day period of exposure leaf injury, ionic permeability, photosynthetic rates, stomatal conductance, transpiration, chlorophyll *a* fluorescence and chlorophyll, soluble carbohydrates and fluoride contents were evaluated. Symptoms of injury by fluoride exposure were visible after 3–4 days in both species. High electrolyte leakage and correlation coefficients between the total ionic permeability and the fluoride content in leaves indicate a fluoride effect on the structural and/or functional integrity of the cellular membranes. Leaf fluoride injuries were quite different in the two species. In *C. gayana* necroses were limited to the leaf tips, while in *P. maximum* damages were observed in the whole leaf, suggesting a higher susceptibility of this latter species to fluoride. Plants also showed a significant decrease in reducing sugar content between 3 and 5 days of exposure to fluoride, but thereafter reducing sugar content increased reaching the content of control plants.

Effects of F on other photosynthetic parameters

While F may affect many metabolic pathways, it is the disruption of one or more specific enzymes in each pathway that results in disruption of the whole pathway. Photosynthetic enzymes that are affected by F include the chloroplast ATPase, RUBP carboxylase/oxygenase, and sucrose synthetase (Parry., 1984; Giannini ., 1985; Quick ., 1989).

Ivinskis and Murray (1984) found that reductions in photosynthetic capacity, chlorophyll a and b and leaf area of grey gum (*Eucalyptus punctata*) were all significantly correlated with leaf F content, F in air and distance from an aluminium smelter. They also found that dusky-leaved ironbark (*Eucalyptus fibrosa*), a species believed to be tolerant of F, showed no significant differences between sites for any of the variables except leaf area. F at concentrations of 190 ppm significantly reduced the *in vitro* photosynthetic capacity of azalea (*Rhododendron* sp.) cultivars (Ballantyne, 1991).

Doley (1988) studied F-induced enhancement and inhibition of photosynthesis in four taxa of *Pinus*. *Pinus elliottii* Englem. var. *elliottii* (PEE) and three varieties of *Pinus caribaea* [var. *caribaea* (PCC), var. *bahamensis* (PCB) and var. *hondurensis* (PCH)] were grown for a total of 125 days under gaseous HF at 0.0, 1.2, 1.8 and 4.3 $\mu g.m^{-3}$. Chlorophyll concentrations in old

needles were consistently lower than those in young needles, but varied less with changes in ambient F concentration. Differences in photosynthetic rates of PCB, PCC and PCH corresponded generally with differences in concentrations of chlorophylls a, b and (a+b). Ambient F concentrations less than about 1 $\mu g.m^{-3}$ appear unlikely to have deleterious short-term effects on chlorophyll concentrations and photosynthesis in these taxa. In wheat seedlings exposed to NaF, the chlorophyll contents increased and then decreased over time (Tomar and Aery, 2000).

Keller (1980) grew Norway spruce (*Picea abies*) cuttings in sand and watered with 100 ppm F during winter until bud break. Watering with NaF significantly depressed the CO_2 uptake of shoots. Exposure to F significantly increased the susceptibility of plants to sulfur dioxide in subsequent fumigation experiments. Respiration was significantly reduced after 24 h, but not after longer exposure times, while photosynthetic oxygen release was significantly reduced at 48 and 91 h but had recovered after 168 h (Zwiazek and Shay, 1988 a).

Rao *et al*., (2008) Studied the response of photosynthetic capacity, chlorophyll a and b concentrations and leaf area in two cultivars of mulberry growing in high fluoride content of soil. Mulberry variety (S54) believed to be sensitive to fluoride, showed reduction in all of these parameters which are significantly, correlated with leaf fluoride contents and fluoride in soil. The same parameters in mulberry variety kanva (M5) which was believed to be tolerant to fluoride showed no significant differences in high fluoride containing soil.

Effects on respiration

One of the earliest manifestations of F toxicity in plants is a change in respiratory rates. Either stimulation or inhibition occurs depending on a number of factors like plant species, concentration of F, age of tissue, length of exposure, pH of culture medium and interaction between various mineral elements and F.

Treatment of leaf tissue with F, either fumigation treatment or tissue culture treatment, may result in a respiratory stimulation or inhibition as measured by oxygen consumption (McNulty and Newman, 1957, Newman 1962).

Low concentrations of NaF significantly increased oxygen consumption and total phosphorylated nucleotides in respiring *Chlorella pyrenoidosa*. Measurements of gas exchange at several pH values indicated that the stimulation was probably related to the undissociated HF concentration in the suspending media (McNulty and Lords, 1960)**.** Miller and Miller (1974) and

Pushnik and Miller (1983) have also indicated that at low concentrations, F may stimulate oxygen uptake, whereas at high concentrations it inhibits oxygen uptake.

F-inhibited tissue respiration may be due to large part to inhibition of respiratory enzymes. Ascorbic acid oxidase, polyphenol oxidase (Lee, 1965) and succinate dehydrogenase, malate dehydrogenase, peroxidase (Lovelace and Miller, 1967), have been shown to be inhibited by physiological concentrations of F. The reasons for F-stimulated respiration are less obvious. Special species of plants have shown an increased use of the pentose phosphate pathway when exposed to F. The activities of glucose-6-phosphate dehydrogenase, cytochrome oxidase, catalase and peroxidase were enhanced in F-injured tissues.

Effects on ATPase and other enzymes

Fluorides have been found to inhibit or stimulate enzymes involved in respiration, photosynthesis and metabolite transport across membranes and other processes effects on vegetation (Miller and Miller, 1974; Miller, 1983; Pushnik and Miller, 1990).

F affects energy metabolism in higher plants by inhibiting the ATP synthase enzymes responsible for ATP formation. The plasma membrane is extremely sensitive to F and should be considered as a critical site of F toxicosis (Giannini, 1987b). This ATP-driven H^+ pump is a primary consumer of cellular ATP and its inhibition may be positively correlated with the observed initial rise in cellular ATP levels reported to occur in higher plants exposed to atmospheric HF. Ballantyne (1984) found that exposure of pea (*Pisum sativum*) shoots to solutions containing 19 ppm F for upto 3 days caused significant increase in ATP levels in the youngest, fully expanded leaves and in entire shoots. This increase occurred before significant decrease in fresh weight, water content or water uptake and stem elongation.

In a study by Giannini (1987b) the effects of F at various concentrations on the plasma membrane ATPase of sugarbeets (*Beta vulgaris*) were investigated. The plasma membrane ATPase was inhibited by lower concentrations (5 mM) of F. The amount of inhibition due to F increased with increasing concentrations of free Mg^{2+} in the reaction medium. The data suggested that F inhibition of the plasma membrane ATPase is at the active site of the enzyme and occurs *via* a magnesium-fluoro-complex. The different P-type transport ATPases could be found in two different conformations named E_1 and E_2. During the catalytic cycle the form E_1 is phosphorylated by ATP. The enzyme form E_2 binds Mg^{2+} and the complex E_2.Mg is phosphorylated by Pi (de Meis and Vianna, 1979; de Meis, 1989; Vara and Medina, 1990). Similar

to the Ca^{2+}ATPase (Coll and Murphy, 1992; Murphy and Coll, 1992) the inhibition of the H^+ATPase of corn roots by F was enhanced by Mg^{2+} and was antagonized by Pi. This suggested that F interacts with the enzyme to form E_2 of the H^+ ATPase.

Facanha and de Meis (1995) studied the inhibition of maize root H^+ATPase by F and fluoroaluminate complexes. Vesicles derived from maize roots retained a membrane-bound H^+ATPase that was able to pump H^+ at the expense of ATP hydrolysis. The H^+ pumping and the ATPase activity of these vesicles were inhibited by lithium F and by the complex formed between F and aluminum. The inhibition promoted by lithium F increased as the $MgCl_2$ concentration in the medium increased from 2 to 20 mM. The inhibitory activity of both lithium F and aluminum F increased as the temperature of the medium was increased from 20 to 35^0C. Further, inorganic phosphate (10-40 mM) inhibited the H^+ ATPase at pH 6.5 but not at pH 7.0, and at both pH values, it antagonized the inhibition promoted by lithium F and fluoroaluminate complexes.

Eastern white pine (*Pinus strobus*) seedlings were grown in controlled environment growth cabinets and fumigated with 0.4 and 1.6 $\mu g.m^{-3}$ HF for 2-28 days. Plasma membranes were isolated from needles of treated and control seedlings and their chemical composition and ATPase activity were examined to determine early effects of HF action. Seedlings treated with HF for 8 days contained plasma membranes with elevated phospholipid: protein and sterol: protein ratios and their plasma membrane ATPase activity was higher than that of control plants. Prolonged, 28-day HF treatment with 1.6 $\mu g.m^{-3}$ level was the only treatment, which produced a drastic inhibition of plasma membrane ATPase activity. The results indicated that the plasma membranes might be among the initial sites of HF injury to plants as well as initial sites of defense reaction (Rakowski, 1995).

In vivo experiments involving HF fumigation of white pine seedlings showed that changes in membrane permeability, greater electrolyte leakage, plasma membrane, lipid composition and increased plasma membrane ATPase activity occured during the early stages of F injury. No changes in mesophyll cell structure were observed before the appearance of visible injury (Rakowski, 1996). In another experiment by Rakowski (1997), when the seedlings were pretreated with 12 h photoperiod to induce dormancy before F treatment, a decrease in ATPase activity was observed after 8 days and but drastic increase was seen after 28 days of HF treated plants. Hence the pretreatment caused a decrease in sensitivity of treated plants and delay in F induced changes.

The tonoplast membrane is the most F sensitive membrane as shown by electron microscopy (Miller, 1993). Tonoplast ATPase displays F sensitivity at

concentration above 30 mM (Giannini, 1987a). The effects of F on the tonoplast type ATPase and transport activities associated with sealed membrane vesicles isolated from sugarbeet (*Beta vulgaris*) storage tissue were examined. This anion had two distinct effects upon the proton-pumping vesicles. When ATP hydrolysis was measured in the presence of gramicidin D, significant inhibition (approximately 50%) only occurred when the F concentration approached 50 mM. In contrast, the same degree of inhibition of proton transport occurred when the F concentration was about 24 mM. Effects on proton pumping at this concentration of F could be attributed to an inhibition of chloride movement, which serves to dissipate the vesicle membrane potential. Valinomycin could partially restore ATPase activity in sealed vesicles, which was inhibited by F and this restoration, occurred with a reduction in the membrane potential. F demonstrated a competitive interaction with chloride-stimulation of proton transport and inhibited the uptake of radioactive chloride into sealed vesicles. When the vesicles were allowed to develop a pH gradient in the absence of KCl, and KCl was subsequently added, F reduced enhancement of the existing pH gradient by KCl. The results were consistent with a chloride carrier that was inhibited by F.

F has also been shown to inhibit the mitochondrial ATPase (Pushnik and Miller, 1983) and CF_1 portion of the chloroplast ATPase (Giannini, 1985). When tissue respiration was enhanced in F tissue (HF fumigation), ATPase activity of mitochondria was increased. This higher ATPase activity could result in increased ADP pools within the cell (Miller, 1993).

Zwiazek and Shay (1988b) reported that 3 ppm F significantly reduced growth (as measured by fresh weight) and acid phosphatase activity and increased total organic acid content of jack pine (*Pinus banksiana*) seedlings. Treatment with NaF *in vivo* altered mitochondrial superoxide dismutase (SOD) activity in moong bean seedlings. NaF at low concentrations (0.1 mM) enhanced SOD activity, but depressed it at high concentrations (1 and 5 mM) (Wilde and Yu, 1998).

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