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# ENVIRONMENTAL FLUORIDE EXPOSURE AND ITS TOXIC EFFECTS ON PLANTS: A REVIEW

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**ABSTRACT: The most reactive electronegative element, fluorine, cannot exist naturally in an uncombined state; it is always found in the form of minerals. It is not an micronutrient for humans, animals, and plants, and when too much fluoride is present in the environment, it poses serious health risks, has a negative effect on the food chain, and disturbs the ecological balance. Depending on the concentration and duration of fluoride exposure, the harm fluoride causes to the environment and plants can be either acute or chronic. Fluoride toxicity in plants influences a variety of physiological, morphological, biochemical, germination, and growth parameters, and it even significantly lowers crop yields, which is economically detrimental to a nation. Human health is highly at risk from consuming fluoride-enriched foods as well as other naturally occurring and man-made sources of fluoride, as high fluoride intake and exposure can alter reproduction, impair growth, and result in dental fluorosis, skeletal fluorosis, mental retardation, and many other disorders. In this review article, a variety of fluoride-related topics have been covered, including the types of fluoride that are present in the atmosphere, their sources, how plants absorb fluoride, and illnesses that may be caused by fluoride toxicity in both plants and animals, most notably humans. The use of fluoride-degrading bacteria created using novel "recombinant DNA techniques" and the use of genetically modified "hyper-accumulator plants" for efficient phytoremediation have both been discussed in relation to defluoridation techniques.**

Keywords: Fluorine, Hyper-accumulator plants, Physiological and biochemical parameters, Defluoridation techniques, Phytoremediation.

## INTRODUCTION

Environment plays an effective role in the existence of healthy life on the planet earth. It is the resultant of all external influences that affect the living organisms and these organisms are basically the product of their environment and their genetic code. The energies and materials are supplied by the environment that governed the DNA mobilization into living organisms.

Environment is also the coupling of biotic (microorganisms, plants and animals) and abiotic factors (water, air) [1]. Living organisms like plants and animals are the short lived entities by which materials and energy flow and ultimately return to the environment. All the species on the earth are dependent on environment that includes the factors like temperature, food, air, water, light, population density, sound and shelter. Environment maintains ecological balance, provides habitat and food for humans and wildlife, and any change in environment can affect the whole ecosystem as well as altering the life cycles of animals and plants [2].

Plants and animals are subjected to a wide range of environmental stresses that limits and reduces the functionality and productivity of these organisms. The major stresses include pollution, drought, salinity and soil erosion, extremes in temperature

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and heavy metals like aluminum [3]. Drought is worse affecting the humanity, agriculture and livestock. In crop yield more annual loss is caused by drought than any other combined pathogens. According to the recent literatures, in crop production, global losses due to drought [4] are approximately 30 billion dollars, in the past few decades. Soil salinity is a global issue that concerns crop output and growth, as well as modern agriculture's long-term sustainability. More than a third of the world's irrigated areas have been damaged by salinization. All of the major staple crops are responsible for the majority of calorie absorption by people (e.g., rice, wheat, and corn), and these crops are unable to complete their life cycle when soil NaCl concentrations [5] surpass 200 mM. Increased anthropogenic exploitation of nature and natural resources causes environmental damage [6] and are a serious problem to agriculture and human life. Pollution is the introduction of harmful substances into the environment. One of the most phytotoxic air pollutants is fluoride, in acidic soils a high level of fluorine reduces crop yield due to decreasing phosphate and increasing Al uptake.

Fluorine, is the most electronegative element and the first member of the halogens, is the thirteenth most frequent element in the earth crust, representing about 0.3 g/kg, and forms minerals and compounds like fluorapatite, fluorspar and cryolite [7,8]. It is not an essential micronutrient for animals, plants and humans, and it can be harmful if levels exceed the WHO limit of 1.5 mg/L [9]. Fluoridation of water is a prevalent technique in nations such as Brazil, Malaysia, Australia, the United States, India, and Vietnam. The majority of European countries do not fluoridate their water. Fluoride concentrations above 1.5 mg/L, may cause skeletal, nonskeletal, and dental fluorosis. Fluoride contamination stress is a growing problem around the world. According to the most recent estimates, fluorosis affects roughly two hundred million individuals worldwide, spread across twenty-five countries. India and China, the world's two most populous countries, are particularly hard hit. Fluoride levels of 1.5 ppm have been documented in several places around the world, including Taiwan, India, Chile, Mexico, Bangladesh and Hungary [10].

Pakistan is a developing country with an estimated population of 220 million, and among them 34% are children [11]. In Pakistan, there is a deficiency of water together with fluoride toxicity. Approximately 1.1 billion people do not have access to safe drinking water, 2.5 billion do not have access to basic sanitation, and more than 5 million people die each year from water-related diseases. In Pakistan, from 29 major cities data, 34 percent of the cities had fluoride levels greater than 1.5mg/L, with maximum values of 24.48, 5.5, 23.60 mg/L in Quetta, Tehsil Mailsi and Lahore, respectively [10]. Excess fluoride in the environment can cause major health problems for plants, humans and animals. This puts human health at risk, has an adverse effect on organism development and growth, and has a detrimental impact on the food chain [12], consequently disrupting the ecological equilibrium. Fluoride compounds can enter the environment as a result of natural processes (e.g., volcanic emissions) or rock, water and soil contamination, and from the wastes of industries. Acute fluoride poisoning (acute fluorosis) in animals is caused by enormous ingestion of rodenticides (sodium fluorosilicate), ascaricides (sodium fluoride), or dental products for human oral use. Severe gastroenteritis, restlessness, salivation, sweating, muscle weakness, anorexia, stiffness, ventricular tachycardia, dyspnoea and clonic

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convulsions are all common symptoms, which are usually followed by depression and mortality. Malnutrition, dental and skeletal abnormalities are common symptoms of chronic fluorosis. Reduced feed and water intake, together with weight loss and low milk production (like in dairy cows) indicate dental lesions and reduced mastication in animals other than humans. The recommended upper limit for plasma fluoride concentration in food-producing animals is 0.2 mg/L, and values ranging from 0.7 to 1.9 mg/L are associated with fluorosis [13].

Fluoride toxicity affects the majority of morphological, physiological and biochemical parameters in plants [14], beginning with germination and early seedling growth and also disrupts the lipid, carbohydrate, and protein metabolism [15] that may result in altered reproduction, and development. Plants absorb F from contaminated soil [16] through their roots or from the atmosphere, hydrolyzes into corrosive hydrogen fluoride that reacts with materials in vapors and aerosols to form non-volatile stable fluorides. This bioaccumulation of fluoride along with heavy metals in edible plant parts is a worldwide problem and a serious threat to human health. But many defluoridation techniques also developed in order to remove fluoride from the environment, including phytoremediation [2, 17].

Tea plant (*Camellia sinensis*) is one of the most popular non-alcoholic beverages in the world, and is a source of Al [18] and F. Its alleged therapeutic potential has helped it gain even more importance as a "health drink." Pakistan has the ability to produce high-quality tea. The National Tea Research Institute (NTRI) is a key player in the production and promotion of tea in Pakistan [19]. Onion (*Allium cepa*) [20] is one of Pakistan's most important commercially valuable crops, and it is widely consumed as a salad, vegetable, and spice. F accumulation pattern in the tissues of onion showed that onion fluoride uptake should be closely monitored and that it should not be planted in fluoride contaminated areas. Rice (*Oryza sativa* L.) [21, 22] is also an essential staple crop, widely grown throughout the Pakistan. Fluoride bioaccumulation in rice grains and straw, might lead to a disaster. At 3 stages of grain development in 3 rice genotypes, like *IR-64, KT* and *GB* (aromatic), the effects of fluoride toxicity may be mentioned. By knowing the importance of these crops in Pakistan and throughout the world, tea plants, onion and rice crops were chosen to investigate F accumulation, absorption, and toxicity in polluted soil.

Fluoride toxicity emerged as a hot topic in the current decade because of its harmful effects on the environment, animals including humans and plants, and so it is necessary to write review articles to give necessary information about this topic and to make suggestions to reduce F contamination.

## FLUORIDE TOXICITY IN THE ENVIRONMENT

**Sources of fluoride:** Fluoride toxicity in the environment is caused by a variety of sources, including groundwater, drinking water, food grown in fluoride-contaminated soils, air from polluted industrial regions, medications, pharmaceuticals, cosmetics containing fluoride compounds, and organo-fluoro chemicals. The addition of fluorine atoms to pharmaceuticals and agrochemicals improves chemical and metabolic stability, bioavailability and potency. During the previous century, the fluoro-chemical [7] sector exploded, and fluorinated pharmaceuticals and agrochemicals became increasingly widespread (between 2010 and 2016, 25–30 %

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of newly launched drugs and 75 % of herbicides contain fluorine molecules). Unfortunately, increased pollution of the environment with inorganic and organic fluorine compounds was a side effect of the aforesaid developments. Two variables exacerbate the difficulty. First, fluorine is an element because fluoride is rare and difficult to incorporate into organic molecules under living conditions. Second, fluorine compounds were often used, before xenobiotic their environmental effects and toxicities were fully understood [23].

**Natural sources of fluoride:** Fluorine is abundant in sedimentary phosphate rock formations and minerals in nature. Fluorspar or fluorite, fluorapatite, and cryolite are common fluorine-containing minerals. The mineral $CaF_2$, (fluorite or fluorspar), has a fluorine concentration of 49 percent and is the most important source of fluorine for industrial uses [24]. Fluorite, also known as fluorspar, is commonly found in combination with quartz, dolomite, calcite and barite. Fluorapatite [$Ca_5(OH, F)(PO_4)_3$], is mined as phosphate rock and relatively common. It has as low inorganic F content (4 % fluorine). Cryolite ($Na_3AlF_6$), on the other hand, is extremely rare (54 % of F). A large number of other silicates, including topaz ($Al_2SiO_4(OH, F)_2$), oxides, carbonates, sulphates, phosphates, sellaite ($MgF_2$), and NaF or villiaumite, include tiny levels of inorganic F in addition to $CaF_2$ and fluorapatite. Mica (layer silicates), amphiboles (chain silicates), apatite, and tourmaline serve as host minerals, as also do clays like montmorillonite, kaolinite, bentonite and apatite. All these contain inorganic F. Fluoride can be found in limestone with tremolite, actinolite, and pyroxene, with F concentrations ranging from 0.4 to 1.2 %. Clay particles admixed with weathered limestone may contain a small amount of fluorine [25].

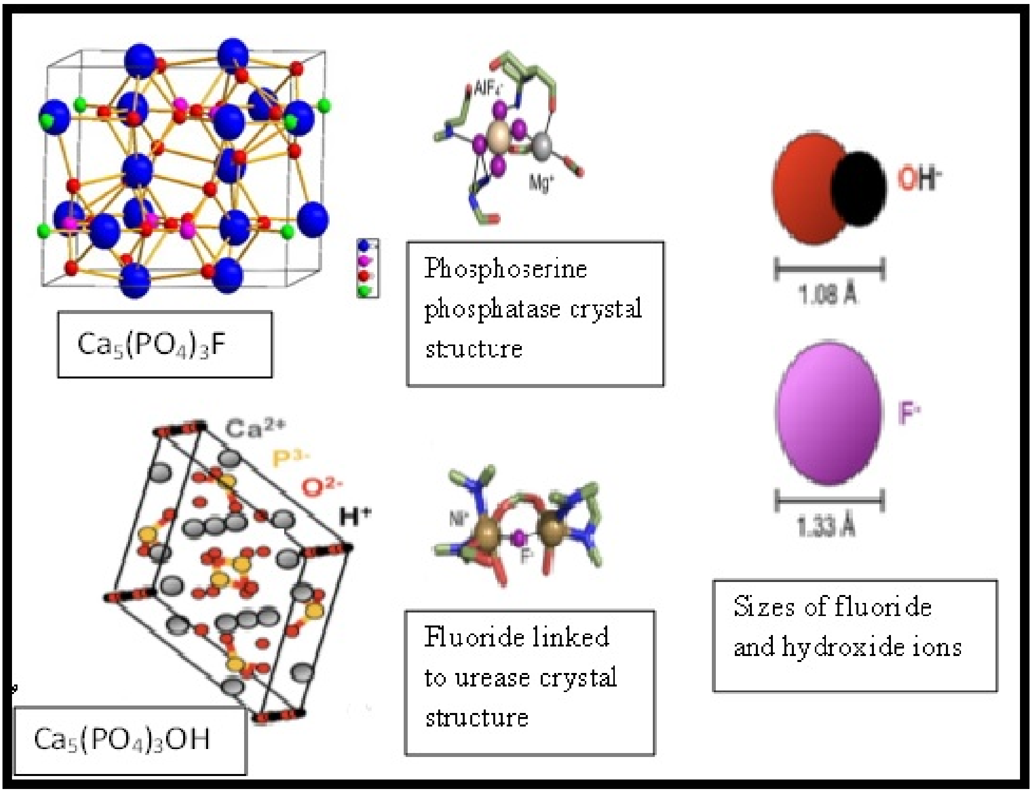

**Figure 1:** *In vitro* fluoride interactions [24].

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## Anthropogenic sources

The use of fertilizers and the deposition of industrial airborne pollutants have a significant impact on the native soil fluoride level in most developed and developing countries. Several anthropogenic factors increase soil fluoride levels [26]. Fluoride toxicity in the environment is caused by anthropogenic activities such as coal combustion, Al smelting, brickmaking, minerals extraction, phosphoric acid and phosphate fertilizer production, and other industrial sources such as fiber glass, steel and enamel industries. Coal combustion has long been thought to be the most significant contributor among the other anthropogenic sources. It appears that brickmaking is currently the most important anthropogenic source of F. With the rapid rise of urban areas in Pakistan, Bangladesh and India, these nations have witnessed a comparable growth in brickmaking, and combined with China [27], they account for over 75% of world brick output. In these countries, as many of the brick kilns in operation are artisanal and unregulated, they are major source of HF emissions.

## Mobility and distribution of $F^-$ in soils

In soil, native F level is heavily reliant on the initial material from which it is derived, and in the soil profile its distribution is a result of processes like soil forming, the most pronounced are the degree of weathering and clay content [28]. Muscovite, biotite and hornblende, which are very common soil minerals, can contain a F in a higher percentage this is why these are the principal sources of $F^-$ in soil. Because $F^-$ is not more found in most soils' surface horizons, it is not a surprise that for F has a low affinity for organic matter.

Fluoride concentrations in organic matter of the surface horizon range from 0.03 to 0.12 ppm. High variability has been documented in practically all the studies of the fluoride concentration of uncontaminated soils. The average fluoride level of soil is 320 ppm worldwide. In average arable soils, total fluoride concentrations vary from 150 to 360 ppm, but can reach 620 ppm. Many studies have related variations in total soil fluoride concentration to soil particle size; higher total fluoride levels are frequently associated with higher clay levels [29]. Total soil fluoride levels are also known to rise with soil profile depth.

Fluoride ($F^-$) makes the strongest bonds with Fe, Al and Ca, but unstable $F^-$ is kept in soil by clay minerals, by Mg, Ca, Al, and Fe compounds. Al and Fe hydroxides and oxides absorb fluoride at low concentrations with a great ability. Fluoride concentrations in natural soil solutions are typically a modest fraction of labile soil fluoride and normal soil fluoride. Fluoride is highly bound in almost all soils, except Al or Fe oxide-poor soils and coarse clay. The ability of various materials to absorb F was ranked in the following order: $Al(OH)_3$ precipitate on bentonite> $Al(OH)_3$ >> hydrated hallosite and dehydrated halloysite > a weakly acidic soil >> kaolinite > gibbsite > alkaline soil > goethite > bentonite and vermiculite. The adsorption capacity of $Al(OH)_3$ for F is quite high. $F^-$ adsorption occurs mostly through exchange with OH groups from $Al(OH)_3$ and basic Al polymers adsorbed on mineral surfaces, rather than with the crystal lattice OH group of clay minerals. The development of somewhat soluble $CaF_2$ and fluoride complexes with Al, Si and Fe in calcareous soils is responsible for the element's modest migration. High quantities of

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exchangeable Na, on the other hand, increased the solubility of F in sodic soils. Chhabra et al. found a linear relationship between water-soluble fluoride and ESP [24].

## IMPACT OF FLUORIDE CONTAMINATION ON PLANTS

Fluoride toxicity is a global issue that has negative consequences for both animals and plants. Although studies on effects of fluoride toxicity on human and animal health have received increased attention in recent years, more research is needed to understand the physiological, biochemical, and molecular basis of fluoride tolerance in agricultural plants. Fluoride in the soil and air has a negative impact on crop development and output, with major implications for global agriculture. So, more molecular study may result in a better understanding of F toxicity stress in plants and the development of resistant genotypes for long-term crop production under abiotic pressures like F toxicity. Fluoride pollution in groundwater is a major source of worry. Many plant species are vulnerable to fluoride pollution which can result in serious harmed. In light of these concerns, F toxicity and the related mitigation techniques have emerged as a significant challenge for agricultural scientists seeking a steady yield under F stress. Appropriate advice for mitigating the hazards of rising F levels in our pastoral systems and developing sustainable practices in the future will only be achievable after more data becomes available [2,31].

Phytin is the substance in plants that is broken down by the enzyme phytase during germination to provide inorganic phosphate to the immature seedling. Fluoride inhibits the phytase enzyme, which prevents the dephosphorylation of phytin compounds in tissues and slows seedling root development during germination. Phytin-derived inorganic phosphates, which are orthophosphate sources, are required for RNA metabolism. One of the variables that may slow the development rate of F-treated seedlings is a scarcity of phytin–derived ortho-phosphates. Crop plant vulnerability to harm from NaF applications to the roots and hydrogen fluoride fumigation of the tops is known to be influenced by nutrient levels of N, P, Ca and S. Fluoride activates the trimeric G proteins in a strong and reversible manner. As a result, fluoride ions trigger several G protein-mediated signal transduction pathways in membrane preparations or crude cell lysates. Purified G-proteins activated by non-hydrolysing “Guanine triphosphate analogues” like $AlF_4$ undergo a conformational shift that favours G subunit separation from the trimeric complex and connection with the effector [6].

Fluoride affects enzymes involved in glycolysis, respiration, photosynthesis, and other chemical reactions. Fluoride has inhibitory and stimulatory effects on the enzyme systems of higher plants. Enzymes that are triggered by divalent cations may be blocked if F reaches hazardous amounts in a plant tissue or organelle, hence numerous investigations of enzymes like enolase and phosphoglucomutase have been conducted. The suppression of enolase (2-phosphoglyceric acid, phosphor-enol pyruvic acid) by F is possibly the most well-known of fluoride's *in vitro* actions, and it was initially investigated in yeast. Fluoride has been shown to have an effect on plasma membrane function. However, relatively few investigations on this critical feature of F toxicity have been conducted. Plasma membrane free sterols: phospholipids, sterols: proteins, and plasma membrane adenosine triphosphatase (ATPase) activity were all greater in white pine seedlings treated with HF for 2 days

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than in control plants. The activity of plasma membrane ATPase was dramatically lowered after 28 days of exposure to hydrogen fluoride at 1.6 mg/$m^3$.

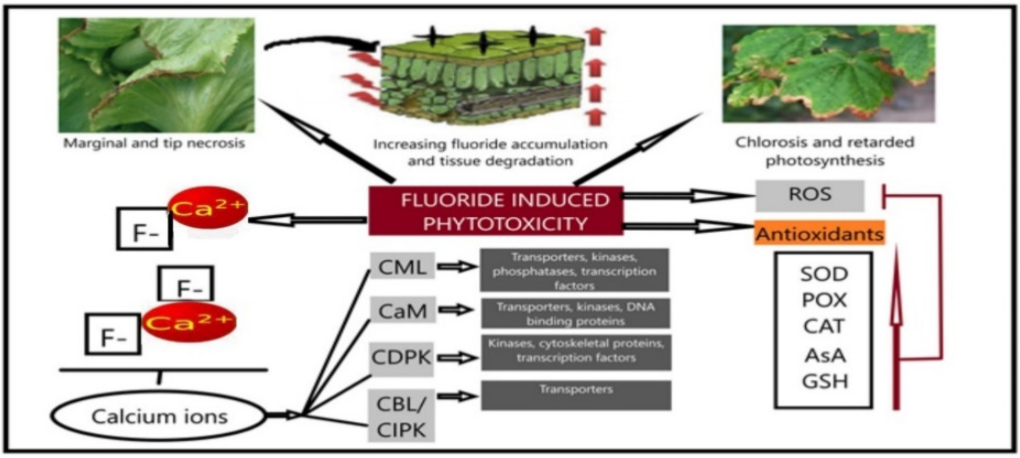

**Figure 2:** Accumulation of fluoride in different plant parts [6].

Despite being one of the most plentiful elements, the phytotoxic effects of F in plants have been understudied. F levels are higher in silt clay loam soils and soils that have been fertilized with phosphate fertilizers. The bioavailability of fluoride in soil is influenced by factors such as pH, permeability and adsorption capacity. F stresses can be edaphic (soil) or atmospheric (in the form of HF) to plants. The stomata absorb the majority of atmospheric fluoride. The roots take up soil Fs, which are then absorbed and translocated to the stems, leaves, and storage organs via an apoplastic mechanism. The precise location of accumulated F within the cell is uncertain. Leaf tip burns, chlorosis and leaf scorch are the most common symptoms of F phytotoxicity. Excess F in sensitive plants slows essential functions such as photosynthesis, signalling, N metabolism, nucleic acid synthesis, and others, while also increasing ROS production [32].

Plants acquire non-enzymatic antioxidants like polyphenols and AsA, and fluoride also boosts the activity of enzymatic antioxidants like CAT, SOD and POX, to scavenge the damaging ROS. Future research should focus on the expression profiles of genes that code for antioxidants. F hyper-accumulators and tolerant species can be screened for phytoremediation in order to control such biohazards. These species may have a high antioxidant content as well as excellent compartmentalization or fluoride ion export [33].

Phytoremediation is a simple and low-cost method of removing F from contaminated soils and water supplies [17]. Fluoride is primarily accumulated in the root system of plants. In contaminated locations, F contamination of food crops might pose a real health risk. Soybean, tea, onion, spinach and rapeseed, all showed dose-dependent yield reductions when soils were polluted with NaF. The lower concentrations use of sodium fluoride in flooding water resulted in significant production increases in pak-choi and lettuce, but output of spinach was encouraged initially and later inhibited in the same study. Lady finger and wheat yields reduced

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by around 50% and 60%, respectively, at larger doses. While addition of fluorine in the form of KF to soil, resulted in dose-dependent drop in triticale yields, rapeseed and yellow lupine yield increased by up to 64 percent and 15%, respectively. The maize and radish aerial biomass increased less dramatically. The yields of narrow leaf lupine increased by 40%, when given 60 mg F/kg. Many researchers looked into the damaging effects of F on plants, finding that it causes oxidative stress, a decrease in chlorophyll content, and changes in proline, soluble sugars, betaine, nitrogen, macro and micronutrient levels [34].

Despite this, indications like apparent damage and a reduction in root and shoot length were not usually seen. In root systems F accumulated largely, with a few exceptions (like tea plants, *Japanese camellia*), according to a review of literature on controlled condition trials. This could be a problem in crops where the roots are edible parts, like radish that accumulated up to 296 mg fluoride per kg dry matter (DM) in the “Irkutsk region of Russia”. In the majority of the crops tested, fluoride buildup in other edible components (such as fruits, leaves, bulbs, seeds, or tubers) was known to be dose-dependent, reaching considerable levels when plants were exposed to high doses of fluoride. As a result, it is hard to estimate the potential risk associated with the ingestion of a contaminated food product in terms of fluoride's non-carcinogenic chronic effects on human health. Apart from the F content in the food itself, the contribution to daily fluoride consumption (and related HI) is also dependent on the amount of the toxic food ingested. In this context, study on the dietary habits of people who live in contaminated areas is crucial in order to better understand the potential health effects of F contamination in farming systems [35].

### Field scale trials

The places where people primarily practice subsistence farming and consume their own food items would be severely affected. In field-scale trials, some authors who conducted surveys on nutrition in local rural communities found that fluoride-contaminated foods play a significant role in the risk of exceeding the permissible dose, increasing the chance of developing fluorosis disorders [36]. Field investigations aimed at determining the F toxicity amount in the food chain, in particular, should be pursued in some severely afflicted places that have previously been overlooked. In reality, field studies largely discovered through research were in Asia (42 % in India and 32 % in China), despite the environmental F toxicity being substantial in South America and Africa, very few field trials were found there. Although, as previously stated, it is impossible to link the F levels reached by crops to a predetermined level of risk, recommendations on the species to be grown in contaminated areas can be based on the degree of F mobility from the soil to edible plant parts, which is commonly measured using the bioconcentration factor (BCF). In contaminated locations, cultivating crops with the lowest BCFs in edible sections is therefore the best option. Planting crops like sugar cane and tea, found to be powerful “fluoride hyper-accumulators”, on the other hand, should probably be avoided [37].

### Influence of factors and appearance of symptoms

Many factors influence the symptoms that occur in plants as a result of F toxicity, including the concentration, temperature, period of exposure, intensity, age of the plant, type of light, their rate of circulation and composition of other gases in

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atmosphere. $F^-$ is carried to leaf edges via the vascular tissue after it has entered the plant in dissolved form. Fluoride accumulation on the superior edge of leaves might spread to the base of leaves, causing mild necrosis. Lower hydrogen fluoride exposure induces prolonged lesions defined through widespread chlorosis and chlorosis along leaf veins in the long run. Acute harm is caused by a high concentration of F, which is characterized by marginal and tip necrosis that progresses to the bases of leaves, or, if quickly absorbed, necrotic irregular patches may occur in the intercostal areas. Physiological, biochemical, structural damage and even cell death according to the cell sap concentration can be caused, when fluoride absorption and translocation to the shoots occurs. According to the histochemical studies of injured plants by fluoride, it has been shown that leaf damage firstly appears in spongy mesophyll and then lower epidermis and is followed by disruption and distortion of the chloroplasts in the palisade mesophyll cells. Due to fluoride toxicity, the upper epidermis is the last to show signs of deformation or collapse [38].

Pigment synthesis in its early stages and the breakdown of the structure of chloroplast may be affected by F. As a result, the concentrations of F at the tips of leaves can reach rather high levels and the early signs of F toxicity are frequently seen at the leaf margins. Photosynthesis and other physiological functions are severely hampered by high levels of F. Necrosis and chlorosis are two evident signs of fluoride's damaging effects on plants. Plant death occurs as a result of both chlorosis and necrosis. Kumar et al. discovered that a 200 mg/kg concentration of F ions reduced chlorophyll content in wheat green leaves, resulting in necrosis and chlorosis [39].

Many studies on the $F^-$ effects on plants have been undertaken by plant fumigation with high concentrations of HF on a large number of plants. $F^-$ is a accumulative toxin in plant leaf, thus it can build up over time. Fluoride has a significant inhibitory effect on photosynthesis and other activities. Fluoride enters plants through the transpiration system from the roots or by the stomata and accumulates in the leaf edges. Fluorine injuries typically manifest as tip and marginal necrosis that spreads inward on broadleaf plants. Plants that have been exposed to high levels of F develop dead regions on tips and margins of their leaves, which become brown or yellow and sometimes become brittle and dry. Drought-stressed plants and plants suffering from salt toxicity show comparable signs. The plant is usually not killed, but the symptoms can be unpleasant.

Fluoride is phytotoxic in most plants because it disrupts a number of metabolic pathways. Fluoride inhibits germination, reproduction, growth, respiration, yield, amino acid and protein metabolism, and photosynthesis by interfering with membranes and stromal enzymes involved in the fixation of carbon dioxide, leading in decreased chlorophyll concentrations. Fluoride inhibits enzymes that need cofactors, like $Ca^{2+}$, $Mg^{2+}$, and $Mn^{2+}$ ions, to function. Fluoride sensitivity appears to be higher in seeds and seedlings than in entire plants. Excess fluoride in vegetation causes obvious leaf damage, fruit damage, and reduction in output.

Necrotic areas, mainly at tips and around the leaf margins, are fluoride toxicity signs in plants. Because few crops have extended cropping seasons, growers irrigate them with fluorinated water for months, increasing danger of toxicity of fluoride. The degree of the injury limited photosynthesis, but the green sections of the leaf

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remained fully functional. Photosynthesis was inhibited for a long time, and recovery was delayed. The actual process by which fluorides harm plants is uncertain.

Due to uneven nutrient intake by seedlings, fluoride toxicity causes a loss in shoot and root length. As the fluoride concentration was increased, shoot length declined progressively, and root biomass was reduced by up to 82.5 % at a fluoride dose of 95 mg NaF/kg soil. Pant et al. found related results for mustard (*Brassica juncea),* wheat (*Triticum aestivum*), Bengal gram (*Cicer arietinum L.)* and tomato (*Solanum lycopersicum*). For Prosopisjuli flora, Saini et al. [40] found that when the amount of NaF in the soil increased, both root and shoot growth reduced. Due to the high concentration of F, Agarwal and Chauhan [32] observed chlorosis and necrosisin the plant, as well as a reduction in root and shoot growth and, as a result, a loss in *Triticum aestivum* yield. Because of lessening metabolic activity in the presence of F, dry weight, fresh weight and % of seedlings declined monotonically with growing F concentration. Further crops, such as mustard, mung, tomato and ladies finger, are more sensitive to F pollution than chilli and maize. According to Bustingorri et al. [41], soybean yield loss reached 30 % at F levels of 375 mg/kg or higher. Raising F above 50 mg/L reduces rice yield. Fluoride is generally found in modest concentrations in vegetables and fruits, between 0.1 and 0.4 mg/kg, although larger quantity of F has been reported in cereals, up to 2 mg/kg. Total soluble sugar and proline content in leaves initially decreased due to slow accumulation of proline during the germination stage, but both increased with rising F concentration due to proline-rich protein synthesis during stress. The increase in sugar and proline levels may be increasing the plant's ability to withstand stress. Total soluble sugars and, especially reducing sugars, in mung bean (*Vigna radiata*) seedlings reduced as F concentration increased. Fluoride decreased the chlorophyll, Mg, Ca, sugar, and starch content of plant leaves. Due to the stress, the protein content of seedling leaves decreased gradually as the F concentration increased [31].

## Mechanism of fluoride absorption in plants:

Fluoride is absorbed by plants from the water, air and soil. The amount of F absorbed determined by type of plant, amount of $F^-$ in air, soil conditions, or irrigation water. A potentiometric approach using “Fluoride selective electrode” was used to define the amount of F in the tissues of *Triticum aestivum* [32]. The stems, leaves, and roots all have different levels of F. Even among cultivars, variable F accumulation in the shoot biomass has been recorded. This is because, despite the roots' passive uptake of F, the variable root-shoot translocation efficiency is critical in the determination of F accumulation in shoots. Individuals with high $Ca^{2+}$ in this location have superior regulation of F ions entry.

$F^-$ is thought to be absorbed through chloride channels, and that a lack of chloride increases the uptake of fluoride ions. F transport could possibly be aided by aquaporins. It is evident that symplastic transport is not possible due to fluoride’s strong electronegativity. The plasma membrane's permanent negative charge prevents fluoride ions from passing through the membrane. However, the apoplastic movement across the cell wall and between cells is prevalent. The endodermis serves as a protective barrier, preventing effective F delivery to vascular tissues and hence reducing the translocation from root-to-shoot. According to Jha et al.[20,25] fluoride retention in *Allium cepa* occurs in the following order, that retention in roots is

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greater than shoots, and lesser in bulbs than shoots. So, the F that reaches the shoots travels in a non-selective manner. This pathway may use an undiscovered method to circumvent the endodermis. F is carried to the leaves through the xylem sap. The amount of F accumulated in the shoots varies by species and is influenced by the amount of F in the rooting medium as well as the velocity of water flow. Chen et al. discovered a collaborative unfavourable effect of Cd and F on the growth of radish plants in a recent study. Within the leaves and at the leaf tips, both Cd and fluoride deposition was identified [42].

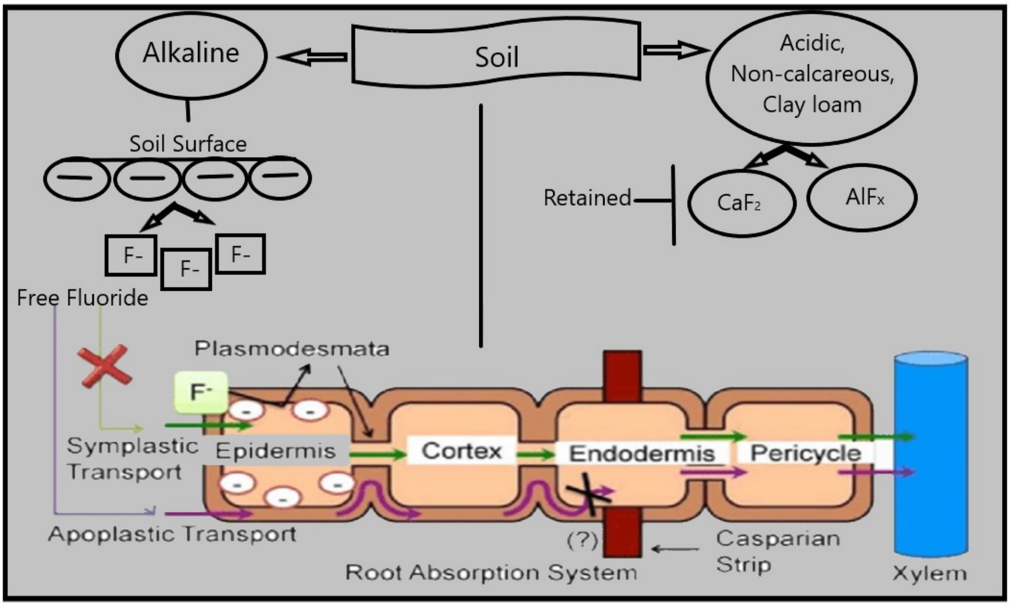

**Figure 3:** Fluoride mechanism in plants through the soil via root system by apoplastic and symplastic transport [31].

## Oxidative stress induced by fluoride and anti-oxidant defenses in plants

Fluoride contamination, the major problem that disrupts the various biochemical and physiological parameters in plants. Plants have evolved complex adaptive systems to cope with stressful environments, but there are still many questions about the mechanisms of F transport, uptake, chelation, degradation, absorption, detoxification, and sequestration, that need to be clarified and elucidated properly. Processes behind fluoride-induced plant harm are still being researched.

The reports on the discovery of hyper-accumulators may give us some useful information about the processes of cellular detoxification and how plants regulate their concentration to reduce stress [43]. Another key goal is to find the varieties of crops that can withstand high fluoride concentrations without accumulating fluoride. The understanding of the techniques used by hyper-accumulators will lead to favorable and useful reactions when these plants are used in fluoride phytoremediation.

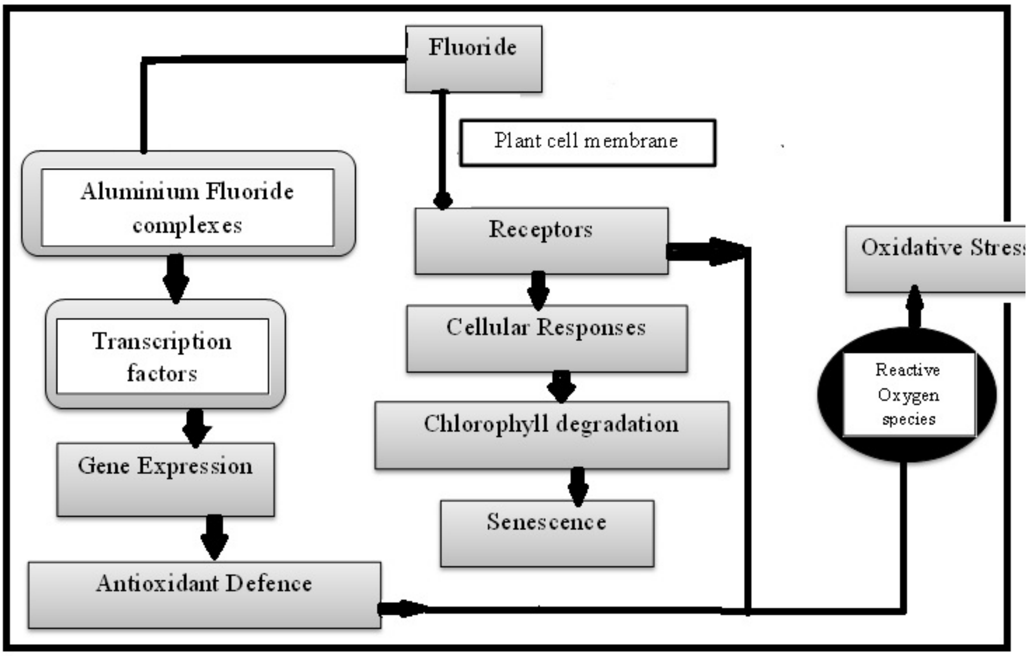

**Figure 4:** Flow chart of fluoride as a stress factor in plants, antioxidant responses, ROS detoxification, signalling to gene expression and oxidative damage [43].

## FLUOROACETATE TOXICITY TO LIVESTOCK AND MICROBIAL DETOXIFICATION

Fluoroacetate (FA; $CH_2FCOOR$) is an organofluorine chemical compound, a colourless salt that is used as a metabolic poison. Because of the 'lethal production' of an isomer of fluorocitrate (FC) [44], it is very hazardous to humans and other mammals by inhibiting the enzyme aconitase in the tricarboxylic acid cycle. Fluoroacetate-producing plants are found all throughout the world, and it is thought that they create this deadly molecule as a defense mechanism against herbivore feeding. In several areas, such as Brazil, Australia and South Africa, cattle ingestion of F results in deadly poisonings, causing considerable economic challenges for commercial farmers. Fencing, poisonous plant eradication, and chemicals that bind the toxin have all been used with varying degrees of effectiveness to protect cattle from the toxicity.

A fluoroacetate-degrading native bacteria was isolated from an Australian cow rumen. All methods to prevent fluoro-acetate toxicity have failed, with the exception of physically limiting access to hazardous plants in the grazing area. Although animal house testing has proven that rumen bacteria developed to hydrolyze the toxin can prevent toxicity, permission for the release of these organisms into the environment is uncertain due to current government regulatory restrictions. As a result, the recent discovery of a naturally occurring rumen bacterium capable of digesting fluoroacetate may provide a biotechnological solution to the problem of pasture animal poisoning. Despite the fact that *Synergistetes strain MFA1* appears to be widespread throughout the digestive systems of animals such as emus, kangaroos and other cattle, its numbers are small, limiting its ability to protect the animal against a deadly

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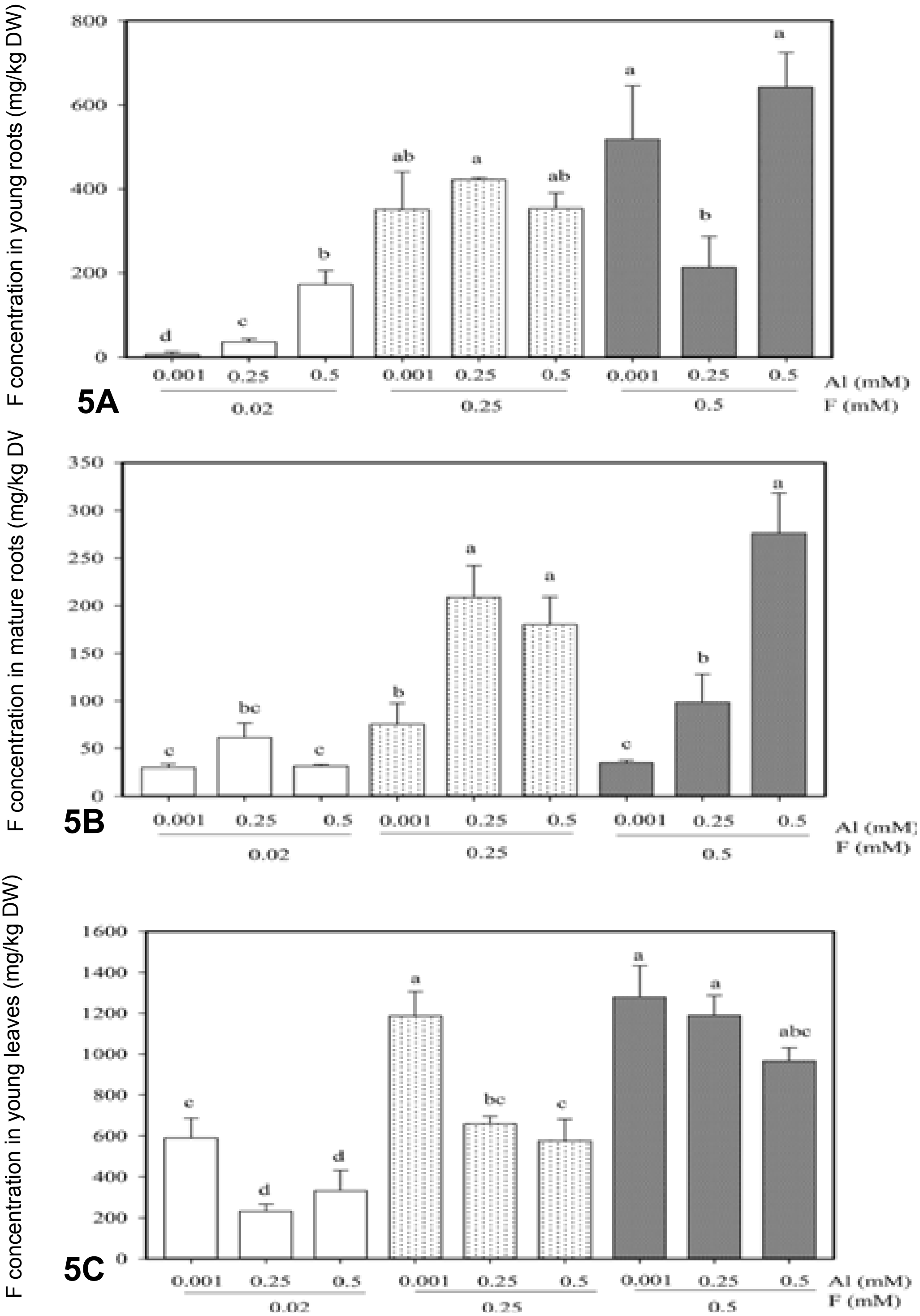
dose of toxin. Other rumen bacteria that can digest fluoro-acetate are likely to be more prevalent, or that they can work together with other rumen microbes to reduce the toxin's full impact. In order to develop a practical strategy to protect livestock from fluoroacetate toxicity [45], more surveys for the presence of other fluoroacetate degrading rumen bacteria, as well as studies on increasing the numbers of these bacteria and expression of the genes responsible for toxin degradation, appear to be a logical approach. According to the recent research, toxicity tolerance by adjusting the rumen bacteria to non-toxic fluoroacetate concentrations adds to the case for rumen detoxification.

## FLUORIDE CONTAMINATION IN DIFFERENT PLANTS

### Tea plants

One of world's most famous non-alcoholic caffeinated beverages, is tea. Literally, it is regarded national drink in Pakistan. It is made from tea plant (*Camellia sinensis*), that has a high fluoride accumulation tendency. The percentage of any element in an infusion of tea taken by the human body is determined by element absorption in the dried leaves of tea plant, the degree of extraction from the leaves into the drink and also by its bioavailability [46]. The physical and chemical characteristics of F, as well as the matrix from which it is absorbed, influence its bioavailability. The majority of water-soluble F in tea infusions is made up of ionic species, which can be free F ions or complexes like Al-F or F-Mn. It is worth considering whether ionic compounds have the same absorption properties as F. Stomach acidity has a big impact on soluble inorganic F absorption. Less soluble inorganic and organic F absorption, on the other hand, is more difficult, with nutritional factors affecting the quantity absorbed. Furthermore, the infusion of tea is high in Mn, Ca, Mg, Al, Zn and $Se^{+2}$ ions, that can bind F and convert it into some less soluble molecules that are excreted in the faeces and urine, lowering F toxicity.

Fluoride bioavailability and excretion are both influenced by tea infusions, and the amount of fluoride in the blood can be reduced. Fluoride intake from consumption of tea is not totally absorbed; both *in vitro* and *in vivo* investigations have shown that F from infusions of dark tea is less bioavailable than in fluorinated water, implying that endogenous chemicals opposed to F are present in infusion of brick tea. The presence of a strong positive connection between urine F content and daily F intake from tea has been demonstrated. According to earlier studies, caffeine has little effect on F absorption, urine excretion or blood levels, while tea-polyphenols, the aflavins, and theanine had no effect on F bioavailability. Polysaccharides and oxidative metabolites of tea polyphenols as the abrownins and the arubigins, according to the results of simulated stomach and small intestinal digestion, can significantly reduce F bioavailability. In addition, drinking habits, particularly the inclusion of milk, should be examined [47].



**Figures 5A–5C:** Fluoride concentrations in tea plant (5A) young roots, (5B) mature roots and (5C) young leaves, produced in a hydroponic experiment with various F and Al doses.The data are means standard deviations (n = 4). At $P<0.05$, different letters indicate a significant difference [47].

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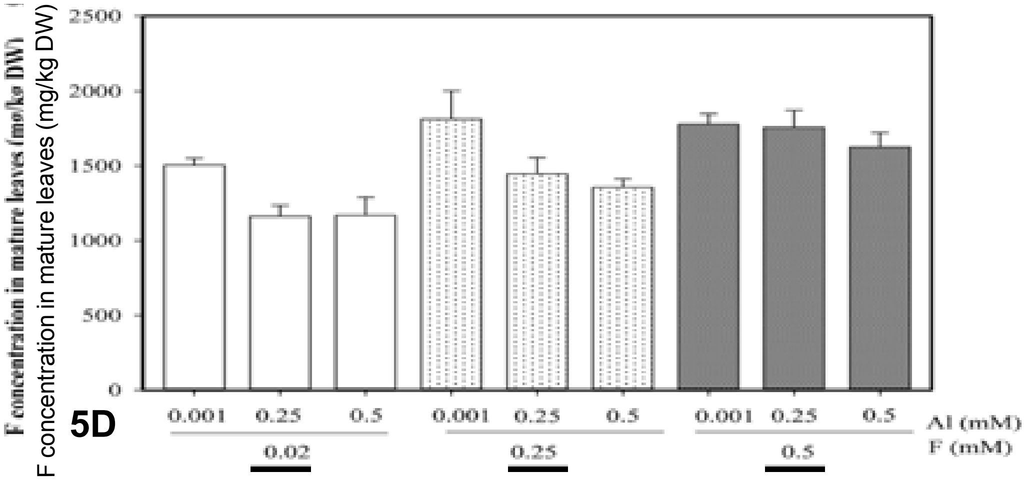

**Figure 5D:** Fluoride concentrations in tea plant (5D) mature leaves produced in a hydroponic experiment with various F and Al doses.The data are means standard deviations (n = 4). At P<0.05, different letters indicate a significant difference [47].

Brick tea, for example, is commonly served with milk, yak butter, or salt, which helps people in northern and western China's border regions reduce fat cholesterol absorption and improve their health. Milk powder lowers F bio-availability and increases faecal F excretion by up to 76.67%. Yak butter has no effect on fluoride availability but increases absorption time, and larger doses of salt increase bioavailability. Tea bags are preferred by 98% of tea drinkers in the United Kingdom, while 96% of black tea consumers add milk. In the presence of meals, F absorption is lowered from about 100 % to 50–80 %, according to some studies [48]. Hydroponically, tea plants were produced with varying levels of F and Al, as well as in a soil pot trial with NaF and acid additives. 19F NMR was used to determine the chemical species of F in solutions of nutrients and the cell saps of leaves and roots. In hydroponic trials, in nutrient solutions in Al absence, F restricted the growth of new shoot and root tips, but Al increased root growth and reduced F toxicity. Aluminium raised the concentration of fluoride in roots but lowered it in leaves.

When Al and F were found in the nutrient solutions, ‘19F NMR’ and ‘Geochem-PC calculations’ confirmed the presence of Al fluoride complexes. Aluminium considerably reduced the nuclear magnetic resonance peak of free F ions in cell saps from leaves and roots. An AlF combination, most likely $AlF^{2+}$, was discovered in the leaf cell sap of plants treated with both Al and F. Fluoride produced leaf necrosis in the soil pot experiment when the leaf Al to F molar ratio was less than 1. In the absence of Al, tea plants are susceptible to F poisoning. Concluding this, by generating Al-F compounds, Al reduces F toxicity in tea plants[49].

## Onion

The onion is one of Pakistan's most valuable commercial crops, and it is frequently consumed as a spice, vegetable and salad ingredient. In a controlled pot experiment, the absorption, accumulation and toxicity effects of F in onion (*Allium cepa L.*) growing on inorganic fluoride (NaF) [50] contaminated soil were examined. Six distinct levels of contamination were achieved by adding 0, 100, 200, 400, 600, and 800 mg NaF/kg to the soil. The levels of F in bulb, root and shoot were 15.8 to 54.3 mgF/kg, 16.3 to 109.1 and 18.6 to 151.6 mgF/kg, respectively. In highly contaminated soils, evident indications of F toxicity such as tip burning and plant death were observed. According to the findings of the current study, F is collected more in the onion shoots than in the onion bulbs, among the edible sections of the onion. The roots, on the other hand, are the key source of accumulation. This demonstrated that the onion plant's tissues include a segregating mechanism for F. Fluoride accumulation pattern in the tissues of onion showed that onion F uptake should be closely monitored and that it should not be planted in F contaminated areas [20].

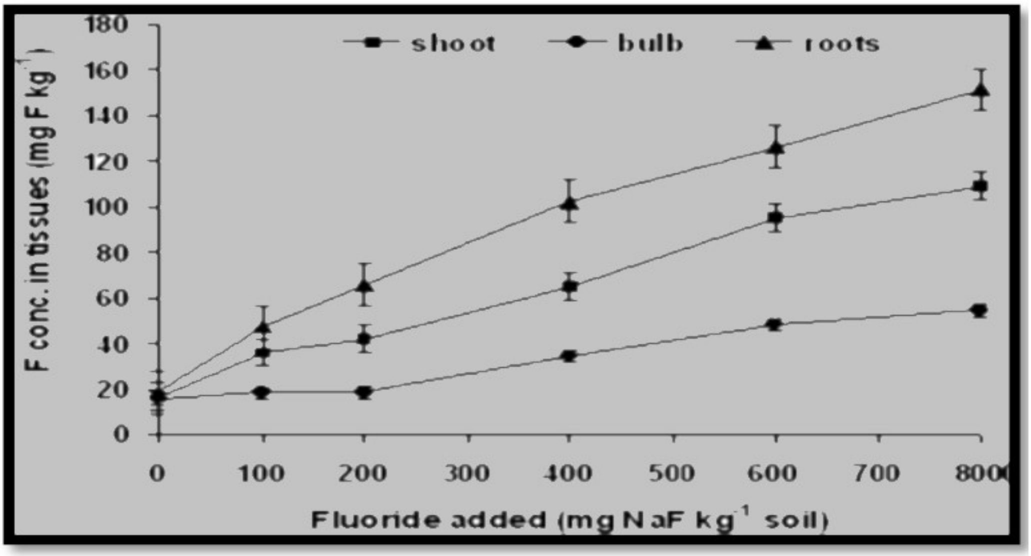

**Figure 6:** Effect of NaF on F concentration in onion bulb, shoot and root: The F concentration is the average of four samples. At the 0.05 probability level, the bars reflect the least significant difference (LSD) between the means [50].

## Rice crop

Rice is a complex carbohydrate that provides energy to almost half of the world's population [51]. In Pakistan, rice is a major income and food crop. After wheat, it is the second most significant staple food crop, and after cotton, it is the second most important exportable product. Rice area increased to 2.9 million hectares in 2017–2018, up from 2.7 million hectares the previous year, resulting in a 6.4% rise in output. Ingestion of F through contaminated food grains has long been regarded to be hazardous to people's health. Fluoride bioaccumulation in rice grains and straw, according to a recent study, might lead to such a disaster. At 3 stages of grain development in 3 rice genotypes [52], like *IR-64, KT* and *GB* (aromatic), the effects of F toxicity were studied. Irrigation with F-polluted water prevented grain hardening in *IR-64* and dramatically raised the oxidative load in aerial biomass, demonstrating the plant's high vulnerability. Fluoride toxicity had no influence on development of seed in *KT* due to the successful maintenance of a strong antioxidant sink, regulated xenobiotic absorption, and high expression of grain-developing genes.

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Because there was no such restricted uptake mechanism after the seedlings died, fluoride accumulation in *KT* straw was much higher than in other cultivars. The fragrant *GB* seedlings were susceptible to F stress in a moderate way. Statistical modelling revealed that, as a tolerant cultivar, *KT* successfully re-programmed the anti-oxidant mechanism during grain development, but *IR-64* reproduction was more vulnerable to F than *GB* reproduction. Because all of the tested rice cultivars are affected with endemic-fluorosis, the overall conclusions have enormous ecological and environmental impact. In the case of *GB* and *IR-64*, the reduced yield and poorer growth physiology might be used as broad indicators of F toxicity. Neither *GB* nor *IR-64* are appropriate for cultivation in fluoride-infested fields, as both cultivars bio-accumulate F at levels far exceeding the allowable limits. Because there was no significant yield loss or physiological inhibition in *KT*, a breeder or farmer would have a difficulty in distinguishing between *KT* cultivated under normal conditions and *KT* exposed to the toxicity of F. This can be a dangerous condition, because consuming F-contaminated straw and grains unknowingly can be harmful to the entire food web.

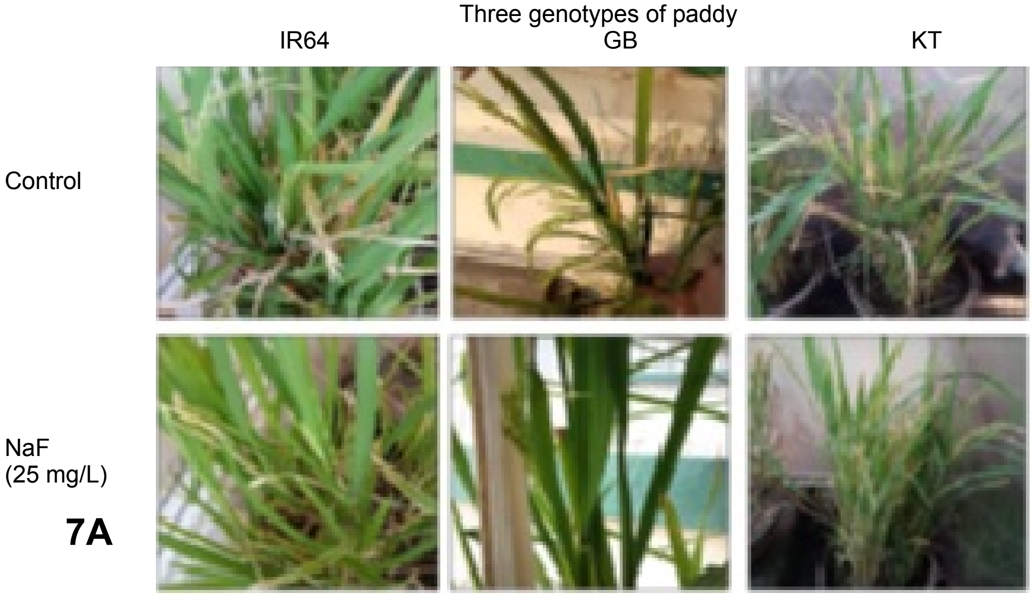
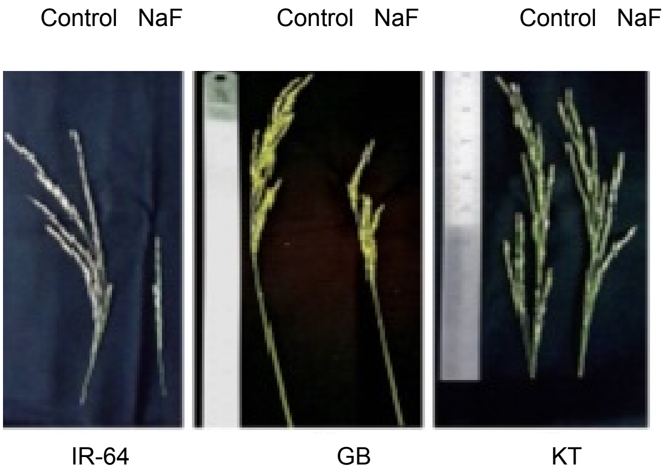
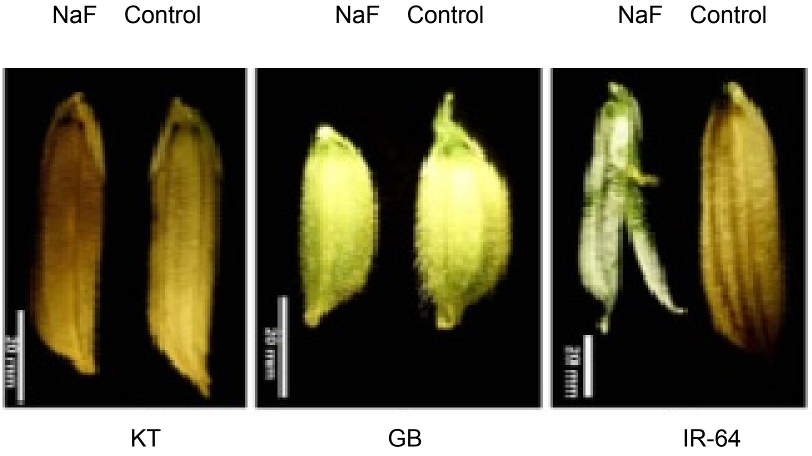


**Figure 7A:** Three genotypes of paddy, IR-64, GB, and KT, with irrigation with double distilled water, control, and 25 mg per L NaF (NaF) throughout their life cycles [53]. 7A: paddy pannicles of seedlings under fluoride toxicity stress.



**7B**



**7C**

**Figures 7B and 7C:** Three genotypes of paddy, IR-64, GB, and KT, with irrigation under fluoride toxicity stress with double distilled water, control, and 25 mg per L NaF (NaF) throughout their life cycles [53]. 7B: paddy pannicle growth patterns and 7C: hardening of individual spikelets.

In comparison to their control counterparts, F stress dramatically reduced *PBL, PL, SBL,* and the number of spikelets per panicle in *IR-64* and *GB seedlings*. When compared to the stressed counterparts of *GB* and *KT*, extreme F toxicity was clearly observed in stressed *IR-64* seedlings, where panicle production was hampered and mature grain development was completely blocked. In stressed *IR-64* plants, the fluid sap that formed within the spikelets did not eventually harden to generate grains, resulting in a complete loss of yield. Fluoride stress lowered the *EG, FGGBD*, and *TGW of GB* grains drastically. The yield of *KT* seedlings was found to be similar in both control and stressed conditions, indicating a high F-tolerant form. Fluoride

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stress has no effect on the yield qualities of mature *KT grains*, though *GL* was lowered slightly in stressed *KT seeds*, so this reduction was determined to be insignificant [53].

The usage of silica nanoparticles with a size of 90 nm can lessen F toxicity in rice plants. Nano-silica was discovered to have a significant impact on rice germination, particularly in the *MTU-1010* variety. Nanosilica was also found to greatly boost the amount of chlorophyll in rice plants. The findings also demonstrated that nano-silica may counteract the detrimental effects of F on root length and had no deleterious effects when used in various concentrations. Silica nanoparticles, in our opinion, might be used as an antidote against F toxicity in plants. More research is needed to establish silica nanoparticles' multifunctional capabilities. More research is needed to establish silica nanoparticles' multifunctional capabilities [54].

## DEFLUORIDATION AND FLUORIDE REMEDIATION

Since the 1930s, a variety of defluoridation procedures have been investigated. The use of these approaches in developing countries have many limitations, necessitating a low-cost solution. Defluoridation techniques including adsorptive measures or reverse osmosis, membrane technologies, are expensive and requires expert operators and continuous maintenance. Furthermore, a complete study of plants that may perform phytoremediation for F uptake in considerable amounts from the environment with the least toxicity, safety, and cost is a long-term strategy to be addressed [35]. The prominent consequences of F on the biosphere are major concerns, and strategies for its safe removal from ecosystem deserve global attention.

Phytostabilization is mediated by plants that have the potential to thrive in the contaminated soils, and other numerous physiochemical strategies have been established. Bio-remediation is safe and novel approach for remediating this contaminant. Absorption, transport, and deposition of pollutants in the aerial portions are parts of the phytoextraction or phytosequestration process. Hyperaccumulators, or plants that can accumulate 0.1 percent or more of pollutants on a DM basis, are commonly used in this method [56]. Phytoremediation with ‘PGPR’ is a new method that solves problems without having any negative consequences. These soil-bacteria break down contaminants into soluble and accessible forms, making phytoremediation easier. Aside from that, the chemical and biological manufacture of metal nano-particles, the establishment of bacterial consortiums, and the employment of transgenics are all gaining popularity in the fight to remove F from the environment with caution and efficiency. Although the above associations have proven to be useful for fluoride handling, more instinctive discovery is required to optimize the plant-microbe interaction, which necessitates a well-balanced combination of various omic-technologies, as well as the expert application of gene-editing and other 'transgenic' approaches to introduce 'foreign genes'.

## CONCLUSIONS

High intake of F from different sources like water, air, soil and other anthropogenic sources are the cause of toxicity in the environment, animals, humans and plants. In the current era, F toxicity is the major worldwide problem. Fluoride causes health concerns like dental, skeletal and nonskeletal fluorosis and other diseases. The environmental exposure of F along with other contaminants like As, Cd and Al cause toxicity when present at high levels. Plant species which are susceptible to F pollution may be greatly affected, resulting in ailments such as chlorosis, necrosis and other physiological and biochemical abnormalities. In light of these concerns, F toxicity and related mitigation techniques have emerged as a significant challenge for agricultural scientists seeking a steady yield from plants under fluoride stress. Tea plants, the major caffeinated beverage, onions widely used as vegetable, and rice crops, a major cash crop and a basic source of carbohydrate, are all consumed greatly throughout the world. In the present review these plants were considered to reveal the effects of fluoride toxicity on their morphology, yield and functionality. To limit the hazards of rising F levels in our agricultural systems and to build sustainable practices in the future, relevant guidelines and F remediation solutions should be made.

## FUTURE PERSPECTIVES

The utilization of diverse biotic agents with specific abilities in treating F toxicity in a more distinct fashion has been better understood as a result of studies on the methods to F remediation. The diverse and unrivalled capabilities of microorganisms and plant varieties open the path for new techniques to improve their effectiveness. Furthermore, strain innovation, development, and selection of ‘new isolates’ will only not open up new treatment options for fluoride, but will also broaden the scope of action. To improve their survivability in the environment and achieve more efficient effects, plant growth promoting microbial consortium and ‘transgenic strains’ for F remediation can be created.

Fluoride degrading bacteria with specialized modes of action can be engineered using new recombinant DNA techniques. Additionally, it is extremely desirable to develop novel strategies for regulation of gene expression, also to apply targeted and random mutagenesis to boost the bio-degrading enzymes activity in these organisms. Aside from that, additional ‘research’ is needed to fully comprehend the metabolic-pathways in ‘transgenic plants’ and bacteria so that their influence and special effects can be discovered when they are used in bio-remediation. Similarly, ‘hyper-accumulator plants’ can be genetically altered for efficient phytoremediation by extracting F from the soil-environment using the phyto-extraction techniques.

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