Chapter 1

General Introduction
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ERICULTURE, THE TECHNIQUE OF SILK PRODUCTION, is an agro-industry, playing an important role in the rural economy of India especially in drought prone areas like Rayalaseema region of Andhra Pradesh. Silk fiber is a protein produced from the silk glands of silkworms. “Silk is the queen of textiles” and the naturally produced animal fiber till today, no other fabric can match it in lust and elegance. The economic advantage of sericulture industry lies in its high employment potential with low investment. One hectare of mulberry creates employment to 12 persons throughout the year. 60% of them are women thereby supporting a greater role for women development. It requires low gestation period and continues to yield for 15-16 years with little expenditure on maintenance. It gives higher returns over 3.5 million people in the country. Sericulture is a cottage industry and provided ample work for the womenfolk in the rural areas in rearing silkworms, while the male members work in the fields. Recently the enforcing of new ideas by research institutions both in mulberry cultivation and silkworm rearing, the industry is now practiced as a main profession and as a major cash crop in the drought prone areas. However, the sericulture industry is facing many problems in Andhra Pradesh specifically, diseases like fungal, bacterial, viral, pests and environmental problems like extreme temperature, humidity, contaminated irrigated waters with fluoride etc. Fluoride is found in substantial amounts in Rayalaseema districts, like Anantapur and Kumool districts where sericulture is being practiced extensively by the small and marginal farmers.

In India fluoride is found in ground water as well as in surface water in hard rock environment comprising granites, gneises, schists and related rock of archean age. Figures 1.1 and 1.2, show the fluoride endemic areas in India and Andhra Pradesh. In Andhra Pradesh fluoride is found in ground water as well as in surface water in hard rock environment comprising granites, gneisses, schists and related rocks of Archaean age. Traces of fluorosis is spread throughout the state and it is
most wide spread in four districts viz., Anantapur, Nalgonda, Karimnagar and Prakasam with concentration of fluoride in groundwater from 3 to 20 mg/l. Nearly 13.5 m people in Andhra Pradesh are at risk due to fluorosis problem.

Fig. 1.1: The distribution of fluoride bearing minerals and prevalence of endemic fluorosis (Teotia et al., 1981)
Fig. 1.2: Fluoride effected areas in Andhra Pradesh (Source: SGWD, Hyderabad, 2004)

The maximum fluoride recorded so far is 25 ppm. In Dharwad, Bellary and Chitradurga districts in the Karnataka state, water has fluoride in the range of 5.4 to 12.8 mg/liter and fluorosis, dental and skeletal forms, is prevalent. In Tamilnadu, the districts of Salem, Tanjore, South Arcot, Dharmapuri and Kanyakumari have high levels of fluoride in water and fluorosis has been reported. In the state of Delhi also, tube well water is contaminated with fluoride. Fluorosis has been reported. In the state of Delhi also, tube well water is contaminated with fluoride. Fluorosis both dental and skeletal forms have been observed in the metropolis and in the villages around Delhi. Dayalpur, Atoli, Chhainsa, Machgar and Sotai village of Haryana have fluoride content ranging-from 1.89 to 3.83 ppm. In Bhatinda, Sangrur and Ferozpur districts in Punjab, about 33 per cent of the population are exposed to the risk of
endemic fluorosis. In Lillya and Lathi taluks in Amreli district of Gujarat, the fluoride content of water samples of tube wells, open wells, ponds and wells on river banks ranged from 0.4 to 8.0 mg/liter. The endemic areas in Rajasthan and Jodhpur, Bhilwara, Jaipur, Nagur, Bikaner, Udaipur, Barmer and Ajmer. In Khanjarpur, Ujera, Madheya Khan ka parva, Sikri of Uttar Pradesh, the fluoride level is found to be 0.62 – 25.0 mg/liter and the incidence of dental and skeletal fluorosis is high. Incidence of dental fluorosis and skeletal fluorosis have also been detected from Bihar, Orissa, Madhya Pradesh and Maharashtra states of India.

Fluoride is found in the man’s natural environment and under normal conditions, is present in the atmosphere, soils, water of rivers, lakes, wells and oceans, rains, snow, in our food and body. It enters in the food chain by several routes but mainly through water. Much fluoride in our environment comes from modern industry and commerce and the natural fluoride chain is supplemented in fluoride by man made chain (Fig. 1.3).

Fig. 1.3 : Fluoride in the Environment through industrial and other man made sources
Fluoride is released into the aquatic environment by a far wider range of sources, and it seems very likely that most bodies of water are contaminated by fluoride to some extent. Fluoride in water is mostly of geological origin and the fluoride affected areas of the world were shown in figure 1.4.

![Fluoride effected areas in the World](Source: SGWD, Hyderabad, 2004)

**Fig. 1.4:** Fluoride affected areas in the World  (Source: SGWD, Hyderabad, 2004)

Across the world waters with high levels of fluoride content are recorded mostly at the foot of high mountains, in areas where the sea has made geological deposits and in granite-gneissic rocks which have fluoride bearing minerals. Some fluoride is present in waters form natural sources. Many minerals contain soluble fluoride, and when ground water passes through such fluoride-bearing rock formations, the water may become contaminated. A few sources, primarily deep wells, contain 1 ppm of fluoride or more. Most surface waters contain less than 0.2 ppm of fluoride, and the majorities are below 0.1 ppm (Dobbs, 1974). The oceans, as the result of eons of leaching of mineral salts from the land, contain from 1.2 to 1.4 ppm of fluoride, about half in the form of fluoride ion and half in the relatively insoluble, magnesium fluoride complex ion (Riley and Skirrow, 1965). Although natural, or “background” fluoride levels in most fresh-water streams are in the 0 to 0.2 ppm range, available data indicate that concentrations above 0.5 ppm, and occasionally as high as 2 or 3 ppm, may be fairly common in water courses contaminated by human activities.
Several human activities result in substantial fluoride input to the aquatic environment. Many of the industries which have fluoride air pollution problems are also the sources of fluoride water pollution. Air pollution control equipment often produces a fluoride laden liquid waste which requires disposal. (Fluoride can be removed from waste water by treatment with like in settling ponds, a form of treatment which can reduce the fluoride content of an effluent stream from more than 5,000 ppm to about 5 to 50 ppm (U.S. E. P.A, 1973). Aggregate figures for all fluoride sources are not available, but the phosphate industry may discharge about 6,000 to 30,000 tons of fluoride into waterways in US annually. (The estimated tonnage of fluoride) The Environmental Protection Agency has proposed standards for the primary aluminum industry which, starting in 1977, would restrict fluoride in wastewater discharge to an average of two pounds per ton of aluminum produced, if all aluminum smelters were currently meeting that standard, fluoride discharges would be 4,000 to 5,000 tons per year from this industry. However, only about one-third of the plants now in operation are presently in compliance, so actual fluoride pollution from the aluminum industry is probably substantially larger (USEPA, 1973). Fluoride discharges from other industries are not negligible, but are probably smaller than from phosphate and aluminum operations.

Another significant source of fluoride after pollution is domestic sewage. Approximately one-half of the communities in the US which have centralized water distribution systems now add fluoride to their water supplies for the partial control of tooth decay (U.S.E P.A, Bethesda, 1970). Provision of fluorinated water for 100 million people requires the addition of approximately 20,000 tons of fluoride to domestic water supplies each year. Most of the water used in urban areas, and thus most of the fluoride added to water supplies, is returned through sewage systems to the aquatic environment.

A study of fluoride levels in sewage in 56 cities of California demonstrated that domestic sewage already contains fluoride, over and above that naturally present in water or added for dental health (Masuda, 1964) Fluoride in human wastes, originating with fluoride in foods, was tentatively identified as the source of the excess. The investigator concluded that fluoride from toothpastes and other sources in world make a negligible contribution, and that no industrial sources were
contributing fluoride to the sewage samples studies. The findings suggest that the total input of fluoride into the environment from domestic sewage is probably more than the 20,000 tons estimated to be added to water supplies in communities where fluoridation of drinking water takes place. Thus, even communities not fluoridating water may release significant fluoride into receiving streams in their sewage.

The same study showed that secondary sewage treatment (biological digestion of wastes) reduced fluoride in the final effluent by; an average of 57 percent, while primary treatment had no appreciable effect on fluoride levels. Even with secondary sewage treatment, however, it was concluded that significant amounts of fluoride persisted in effluents. Fluoride is present in phosphate fertilizers, and some fluoride may be carried into surface waters in runoff from agricultural lands. It is also likely that some portion of fluorides emitted into the air is eventually carried by precipitation into surface waters (Marier and Rose, 1971). While these sources may be significant, good quantitative estimates of the magnitude of fluoride input to the aquatic environment by these routes are not available.

**Soil fluoride**

Because fluoride is a common constituent of several relatively abundant minerals, most soils contain this element. Fluorides are dissolved by passage of water through fluoride containing rocks. There are three most common sources of fluoride (i) Fluorspar (which contain calcium fluoride), (ii) Cryolite (fluoride combined with aluminum and sodium), (iii) Appatite and Rock Phosphate (a calcium phosphate fluoride complex (Maier, 1963). Different soils contain different concentrations of fluoride. Heavy soils (2640 ppm fluoride) tend to higher than sandy soils (76 ppm). Fluoride reach in soils through weathering of rocks, rain water, waste water (waste run off and fertilizer). High fluoride soils generally have high CaCO₃ (90 % F bound).

**Fate of Fluoride in Soils**

More than 90 percent of the natural fluoride content of soils is insoluble, or tightly bound to soil particles (Marier and Rose, 1971). Most soil samples show lower fluoride content near the surface than at depths of a few feet, indicating that the soluble fraction of fluoride may be removed from the surface by water seeping into
the pound. It appears, therefore, that under normal conditions very little fluoride is available for uptake by plants even in soils that may be relatively rich in fluoride.

Research findings differ on the degree to which fluoride added by pollution or fertilization is available for uptake in the plant roots. When soluble fluoride compounds (for example, sodium fluoride) were added to soils in concentration of 150 ppm or more during one experiment, significant uptake by plants occurred and other experiments showed that a substantial amount of fluoride was removed from polluted soils by water (Gisiger, 1968). On the other hand, it has been found that as much as 90 percent of fluoride from fertilizers and air pollution may remain in the soil (Oelschlager, 1971) another report showed that some soils, especially those with relatively high calcium content, were very effective in fixing fluoride, with the result that little was available for plants to incorporate (Macintire, 1950). The range for most normal soils is 100 to 300 ppm, but levels of up to 8,300 ppm have been found in heavy clay soil. Additional sources of fluoride input to the soil may be present in may localities. Air pollution can lead to a substantial increase in soil fluoride content, both through fallout of particulate fluorides and through the absorption of gaseous fluorides in rain and snow (Hiuchan, et. al, 1964). Phosphate fertilizers may contain 0.5 to 4.0 percent fluoride by weight as an impurity. One investigator calculated that fertilizer application in Germany were adding from 7.0 to 17.6 pounds of fluoride each year per acre of land and fertilized (Oelschlager, 1971). This is compared to 1.8 pounds per acre of fluoride added to the soils in his study area by air pollution, and to values of 6.1 to 19.2 pounds for each acre input from air pollution in similar studies. In the US, 5 million tons of phosphate fertilizers were applied to soils in 1973. If it is assumed that the average fluoride content of the fertilizer was 2 percent by weight, this represents an input of 100,000 tons of fluoride to US soils. Additional fluoride input to soils may occur when fluoride-containing waters are used in irrigation. No quantitative estimates are available for the magnitude of such contributions to fluoride contamination of the soil.

However, it seems very likely that a number of soil characteristics, as well as other environmental factor, can have a marked influence on the availability of fluoride to plants. For example, fluoride is more readily available in sand or acidic soils than in high-clayey as soils (Gisiger, 1963). Also, a relationship exists between the type of
nitrogen fertilizer applied and the toxicity of fluoride to crops (Jurkowska, 1971). The use of certain boron-containing fertilizers leads to a dramatic increase in the accumulation of fluoride in the leaves of fruit trees (Bovay, 1969).

Plants

Fluoride occurs naturally in plants, but its presence has attracted attention primarily in certain industrial areas where concentrations are elevated above normal by accumulation from the atmosphere (Yang and Miller, 1963). Concentrations of fluoride found in various components are shown in Table-1 (Miller, 1993).

Table 1.1: Fluoride in Natural Environment

<table>
<thead>
<tr>
<th>Component</th>
<th>Description of fluoride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>76 ppm in sandy soil. 2640 in heavy clay. High F Soils generally have high ( \text{CaCO}_3 ) (90% F bound)</td>
</tr>
<tr>
<td>Air</td>
<td>Average fluoride concentration 0.04 -1.2 ppm. Less than 8% urban areas and 0.2% rural areas have over 0.1 ppm F.</td>
</tr>
<tr>
<td>Water</td>
<td>Average levels 0.1 to 1 ppm. India has waters with high fluoride (low calcium) up to 25 ppm. America has waters (high calcium) up to 25 ppm.</td>
</tr>
<tr>
<td>Vegetation</td>
<td>Normal levels 1015 ppm. Tea may have 400 ppm.</td>
</tr>
<tr>
<td>Terrestrial Mammals</td>
<td>Bone less than 1000 ppm, soft oranges less than 5 ppm.</td>
</tr>
<tr>
<td>Ocean</td>
<td>Ionic fluoride 0.4 – 0.7 ppm</td>
</tr>
<tr>
<td>Seafood</td>
<td>High in fluoride: Mackerel 27 ppm (Fresh weight), fish protein 761 ppm (dogfish)</td>
</tr>
<tr>
<td>Entire food chain,</td>
<td>0.4 – 0.8 mg/day (USA fluoride-free areas), 1.0 mg/day (Czechoslovakia) 1.0 mg/day (World average)</td>
</tr>
<tr>
<td>Amount consumed per day by humans</td>
<td></td>
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</tbody>
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ppm – Parts per million  ppb – parts per billion


Fluoride and its effects on the physiology and metabolism of plants have been the subject of various reviews (Mc Cune and Weinstein, 1971; Marrier and Rose, 1971 and Miller et al., 1983). Despite a few reports of beneficial effects of fluoride on plants (Brewer, 1966). It is considered as an essential mineral nutrient. Acute foliar symptoms by fluoride are expressed by marginal necrosis on broad leaves or tip burn of conifer needles. Plant tissues that accumulate an injurious amount first turn yellow, then some shade of tan to brown or reddish brown, often with a narrow, darker band at the boundary of living tissue. Slow accumulation of fluoride over days or weeks leads to chlorosis symptoms of chlorosis at leaf tips and margins. This is the
more common situation because pollution control devices and tall smoke stacks have greatly diminished at ground level near many pollution source. Plant species and individual plants within species vary widely in tolerance to fluoride. The present review hereunder, deals with the fluoride effect on organisms on experimental and field. As it is highly difficult to review the literature on all the organisms, a brief review on the toxicological effects of inorganic and organic fluoride compounds is given below:

Bacteria

Several investigators have exposed a variety of bacteria and microscopic animal species that live in freshwater to a range of fluoride concentrations extending well above those likely to be encountered in streams, without any demonstrable toxic effects (Wantland, 1956; Grune and Sload, 1955 and Vajdic, 1966). Many species have yet to be tested, however, the criteria for evaluating toxicity were not sophisticated. Masuda (1964) suggested bacterial digestion of sewage removes much of the fluoride content of the effluent and also suggests that some bacteria may accumulate fluoride from water. The importance of bacteria as a basic element in food chains makes it important to learn more about the capacity of microorganisms to bioconcentrate this contaminant.

Algae

The single-celled green alga *Chlorella* showed a 37 percent reduction in growth over 48 hours when exposed to a 2 ppm fluoride solution; (Smith and Woodson, 1965) and reported that 43 ppm was lethal to another alga, *Scenedesmus* (McKee and Wolf, 1963). Few other data on toxicity of fluoride to aquatic plants are available, but several studies suggest that water plants can accumulate the element. Five-day exposures to 100 ppm led to a 50-fold concentration of fluoride by aquatic plants, and fourteen days at 20 ppm produced a 38 fold increase (Marier and Rose, 1971). Water hyacinths absorb fluoride efficiently at concentration above 10 ppm, and to a much lesser extent at lower levels (Rao et al., 1973). Several species of marine algae (exposed to 0.5 to 0.7 ppm) contained 2 to 22 ppm fluoride. The alga *Cladophora*, however, showed no significant fluoride buildup after seventy-two days in sea water with 52 ppm fluoride (Hemens and Warwick, 1972). One Russian study found an average fluoride content of 40.5 ppm in samples of several freshwater plants
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(Danilova, 1944) and other studies strongly suggest that aquatic vegetation accumulates fluoride (Mun, et al., 1966). However, the evidence as a whole is still too fragmentary to provide a clear or systematic picture of the capacity of fluoride buildup in aquatic plants.

McNulty and Lords (1960) exposed the green alga *Chlorella pyrenoidosa* to hydrogen fluoride in 110-min tests. Significant increases in oxygen consumption and total phosphorylated nucleotides were observed at fluoride concentrations of 2, 20 and 200 mg/litre (0.105, 1.05 and 10.5 mmol/litre). Nichol et al. (1987) found no effect of fluoride at concentrations ranging from 5 to 150 mg/litre at a variety of pH levels (5.9–8.0). LeBlanc (1984) calculated 96-h EC50s, based on growth, to be 123 and 81 mg fluoride/litre for the freshwater green alga *S. leopoliensis* and the marine alga *Skeletonema costatum*, respectively.

Antia and Klut (1981) exposed five euryhaline phytoplankton species to fluoride concentrations ranging from 50 to 200 mg/litre (14–15% salinity). The halophyte *Pavlova lutheri* was 35–50% inhibited at fluoride concentrations of $150$ mg/litre. The dinoflagellate *Amphidinium carteri* was 20–25% inhibited at 150 mg/litre and more than 90% inhibited at 200 mg/litre. Hekman et al. (1984) studied the effect of dissolved fluoride concentrations of up to 150 mg/litre on six phytoplankton species.

*S. leopoliensis* growth ceased for a period followed by growth at a reduced rate at 50 mg fluoride/litre; the threshold for growth effects and inhibition of photosynthesis in this species was 25 mg/litre. Nichol et al. (1987) found that fluoride concentrations of $100$ mg/litre (5.2 mmol/litre) caused a growth lag in at neutral pH. Joy and Balakrishnan (1990) studied the effect of fluoride on the diatoms *Nitzschia palea* (freshwater) and *Amphora coffeaeformis* (brackish water) during 96-h exposures. Significance enhancement of growth, compared with controls was observed at fluoride concentrations ranging from 30 to 110 mg/litre with *N. palea* and at concentration 70 mg/litre in *A. Coffeaeformis*. *A. coffeaeformis* did not show significant growth differences form controls at fluoride concentrations of 90 and 110 mg/litre. Van Wensem and Adema (1991) studied the effect of potassium fluoride (32.3–3230 mg fluoride/kg [1.7–170 μmol fluoride/g]) on Carolina poplar (*Populus*
canadensis) litter. The authors concluded that fluoride seems to be toxic for microbial processes at concentrations found in moderately fluoride polluted areas.

Rao and Pal (1978) found a positive correlation between concentrations of fluoride in soil and soil organic matter content at eight sites near an aluminum factory in India and inferred that fluoride decreased the activity of soil microorganisms responsible for litter decomposition. Tscherko and Kandeler (1997) studied the influence of atmospheric fluoride deposits on soil microbial biomass and its enzyme activities near an aluminum smelter at Ranshofen, upper Austria.

Wang (1986) exposed the common duckweed (Lemna minor) to fluoride and calculated an EC50, based on a reduction in frond growth, to be >60 mg fluoride/litre. Fluoride toxicity to terrestrial plants has been studied extensively (Weinstein, 1977). The induction of fluorosis has been clearly demonstrated in laboratory, greenhouse and controlled field plot experiments (Weinstein, 1977; Hill and Pack, 1983; Staniforth and Sidhu, 1984; Doley, 1986, 1989 and McCune et al., 1991). Weinstein and Alscher-Herman (1982) reviewed the physiological responses of plants to fluoride. They concluded that calcium and magnesium play a central role in the responses of plants to fluoride. A detoxification mechanism appears to consist of the sequestration and insolubilization of fluoride by reaction with calcium.

A large number of papers published on fluoride toxicity to plants concern greenhouse fumigation with hydrogen fluoride. Wolting (1975) observed leaf necrosis on freesia (Freesia sp.) cultivars during continuous fumigation at 0.5 μg fluoride/m3 for 5 months and during intermittent fumigation with 0.3 μg fluoride/m3 (6 h/day, 3–4 times/week) for 18 weeks. Long-term greenhouse experiments (2–10 growing seasons) were conducted to determine the effects (necrosis, photosynthesis and growth) of hydrogen fluoride on 16 varieties of flower, fruit, vegetable and forage crops (Hill and Pack, 1983).

Murray (1984b) exposed grapevines (Vitis vinifera) to hydrogen fluoride concentrations of 0.07 (control), 0.17 and 0.27 μg fluoride/m3 for 189 days. Both chlorophyll a and total chlorophyll were significantly reduced by fluoride exposure. Doley (1986) fumigated three varieties of grapevine with hydrogen fluoride 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 at 1.5 μg/m3 of fluoride concentration. 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 fluoride/m3.

Suture red spot is a serious physiological disorder of the peach (*Prunus persica*) fruit. In open-top field chambers, hydrogen fluoride levels causing an induction of this disorder were 0.3 μg fluoride/m3 in continuous exposures of 80 days and 1.0 μg fluoride/m3 in intermittent exposures for three 6-h periods each week for 9 weeks (MacLean *et al.*, 1984b). Wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) exposed to hydrogen fluoride (0.38 μg/m3) for 90 days showed no effect of treatment on yield. There was, however, a significant increase in the grain protein concentration of fluoride-exposed barley plants (Murray and Wilson, 1988c). MacLean *et al.* (1984a) exposed wheat (*T. aestivum*) and two sorghum (*Sorghum* sp.) hybrids to hydrogen fluoride at concentrations ranging from 1.6 to 3.3 μg/m3 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.

Madkour and Weinstein (1988) found that hydrogen fluoride at nominal concentrations of 1, 3 and 5 μg fluoride/m3 inhibited the active loading of [14C]sucrose into the minor veins of soybean (*Glycine max*) leaf discs during 8- to 11-day exposures. Similar results were obtained for both controlled-environment and field conditions. Several species of eucalypt (*Eucalyptus* spp.) have shown sensitivity to fluoride. Murray and Wilson (1988b) exposed *E. tereticornis* to hydrogen fluoride (0.38 μg fluoride/m3) for 90 days in open-top chambers. Fluoride significantly reduced leaf surface area and weight in mature and immature leaves. The same significant effects on immature leaves were noted for marri (*E. calophylla*), tuart (*E. gomphocephala*) and jarrah (*E. marginata*) at 0.39 μg fluoride/m3 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 endpoints were unaffected (Murray and Wilson, 1988a). Coniferous trees have also been identified as sensitive plant species. In field exposure chambers, significant dose–response relationships
were observed between hydrogen fluoride 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).

Airborne fluoride 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 hydrogen fluoride (2 µg fluoride/m³) in a controlled chamber led to significantly larger hypocotyl lesions after inoculation with either *Rhizoctonia solani* or *Phytophthora megasperma*.

Several studies on fluoride have been carried out in culture media. Belandria *et al.* (1989) studied the effect of sodium fluoride on the germination of lichen ascospores. They found that 20% of the *Xanthoria parietina* spores were able to germinate in the presence of 19 mg fluoride/litre (1000 µmol/litre). Ballantyne (1984) found that exposure of pea (*Pisum sativum*) shoots to solutions containing 19 mg fluoride/litre (1 mmol/litre) for up to 3 days caused significant increases in ATP levels in the youngest, fully expanded leaves and in entire shoots. There was evidence from experiments on chlorophyll *a* fluorescence of a reduced ability to develop or maintain a transthylakoid proton gradient in chloroplasts containing elevated levels of fluoride (Boese *et al.*, 1995). Fluoride at concentrations of 190 mg/litre (10 mmol/litre) significantly reduced the *in vitro* photosynthetic capacity of azalea (*Rhododendron* sp.) cultivars (Ballantyne, 1991). Stevens *et al.* (1998) grew tomato (*Lycopersicum esculentum*) and oat (*Avena sativa*) plants in nutrient solutions (12–13 days) at nominal sodium fluoride concentrations ranging from 1 to 128 mg fluoride/litre. Aluminum–fluoride complexation increased fluoride uptake and toxicity in oats relative to the free fluoride ion; shoot and root dry weights were significantly limited at A1F2⁺ concentrations of 11–22 µmol/litre and AIF2⁺ concentrations of 126–357 µmol/litre (Stevens *et al.*, 1997). Ratsch and Johndro (1986) calculated EC₉₅, based on inhibition of root growth, for lettuce (*Lactuca sativa*) plants exposed to fluoride. For the 115-h paper substrate method, an EC₅₀ value of 450 mg fluoride/litre was found, and for the 5-day solution method, the EC₅₀ was 660 mg fluoride/litre. Cooke (1976) studied the effect of fluoride (200 mg/litre) on common sunflower (*Helianthus annus*) seeds grown in sand culture. Keller (1980) grew Norway spruce (*Picea*...
abies) cuttings in sand and watered with 100 mg fluoride/litre during winter until bud break. Watering with sodium fluoride significantly depressed the carbon dioxide uptake of shoots. Zwiazek and Shay (1987) grew jack pine (Pinus banksiana) seedlings in sand culture at 3 or 15 mg fluoride/kg dry weight.

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, 1988a). Zwiazek and Shay (1988b) reported that 3 mg fluoride/kg significantly reduced growth (as measured by fresh weight) and acid phosphatase activity and increased total organic acid content of jack pine (P. banksiana) seedlings.

In pot experiments, Singh et al., (1979) grew rice (Oryza sativa) plants in soil (pH >9.4) amended with 25–200 mg fluoride (added as sodium fluoride); the rice plants were harvested, and the soil was subsequently sown with wheat (Triticum aestivum). The authors found that the critical water-extractable fluoride concentration in soil for grain yield in wheat was 22 mg fluoride/kg, which related to a fluoride content of 35 mg/kg in mature wheat straw. Elrashidi et al. (1998) found that an application of 100 mg fluoride/kg 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 growing on alkaline soil (pH 7.5) were unaffected at 1000 mg/kg. The authors found no clear influence of phosphate (50–550 g/kg soil) on the adverse effect of fluoride on dry matter yield.

Fluoride may be significantly increased in air, water, food, soil, vegetation and mammals by industrial sources. Fluoride pollutants affect the health of plants near sources of emission. Most fluoride pollutants are released during the manufacture of various products for which raw materials are mined from earth. Plant injury by fluorides is not common and severe near sites where phosphate fertilizer and aluminum are produced because these processes employ fluoride-rich materials (Chang, 1978). Fluorides are also distributed in the atmosphere originating from heavy chemical industries which manufacture phosphate fertilizers, aluminum products, fluorinated hydrocarbons, fluorinated plastics etc. Phosphate rocks contain 3.5 to 4 percent fluoride are used in manufacturing phosphate fertilizers. Naturally,
the dust of fluoride containing soils, the gases emitted in areas of volcanic activity also release a considerable amount of fluoride in the atmosphere. The average fluoride content in the air is 0.04-1.2 ppb. 90% of the air samples taken in an industrial city in the federal Republic of Germany during 1965 and 1966 contained fluoride concentration of 0.5 to 3.8 ug per m3 (National Research Council, 1971).

Aluminum smelters, brickworks, phosphorus plants and fertilizer and fiber glass plants have all been shown to be sources of fluoride that are correlated with damage to local plant communities. Klumpp et al. (1994, 1996a) reported that plants downwind of fertilizer industries in Brazil showed damage (necrotic bands or tip burn). Bioindicator plants have revealed that severe damage corresponded to high leaf fluoride concentrations (no statistical analysis performed). Klumpp et al. (1996b) found a highly significant linear regression between leaf damage and fluoride accumulation in \textit{Gladiolus} plants; the plants developed typical fluoride-induced leaf lesions. Murray (1981) found that plant communities near an aluminum smelter showed differences in community composition and structure due partly to variations in fluoride tolerance.

Lichens have been used widely as biomonitors of fluoride pollution. LeBlanc et al., (1971) transplanted epiphytic lichens from an unpolluted area to various distances from an aluminium factory. Lichens accumulated 600–900 mg fluoride/kg during periods of 4 or 12 months within 8 km of the factory compared with 70 mg/kg at a control site. Gilbert (1985) monitored lichens near an aluminum smelter during its 11-year operational life. Epiphytic lichens were severely damaged (>50% injury) over a 25-km2 area close to the smelter. Perkins and Millar (1987) studied the effect of airborne fluoride emitted from an aluminum works on previously unpolluted assemblages of saxicolous lichens. Prior to emissions, lichens contained a mean concentration of 16 mg fluoride/kg. Fruticose (shrubby) lichens, such as \textit{Ramalina} species, were the most sensitive to fluoride emissions, with lichens containing >100 mg fluoride/kg showing severe damage. Loss of lichens decreased with increasing distance from the works.

Bunce (1984) reported that the growth reduction of forest (2800 m3/year) estimated for the years from the establishment of an aluminium smelter (Kitimat,
British Columbia, Canada) to 1974 declined by 78% for the period 1974–1979. The effects of fluoride emissions from a phosphorus plant (the plant closed in 1989) in Long Harbour, Newfoundland, Canada, on the conifers balsam fir (*Abies balsamea*), black spruce (*Picea mariana*) and larch (*Larix laricina*) were monitored at sites downwind from the plant during the summer of 1982 (Sidhu and Staniforth, 1986). Mean fluoride concentrations ranged between 11.4 μg/m3 at a distance of 1.4 km from the source and 0.08 μg/m3 18.7 km from the source. At the closest site, seed production in balsam fir, black spruce and larch was impaired by 76.4%, 87.4% and 100%, respectively, compared with controls.

Reproductive and vegetative characteristics of raspberry (*Rubus* sp.) and blueberry (*Vaccinium* sp.) were monitored at six sites downwind from the plant. Within 1.4 km of the plant, flower mortality was 89% for blueberry and 78% for raspberry, compared with 27% and 26%, respectively, for a control site; there were also significant decreases in size, number and dry weight of fruit. Fluoride concentrations in foliage were 403 mg/kg for raspberry and 216 mg/kg for blueberry, compared with 8 and 9 mg/kg, respectively, for the control site (Staniforth and Sidhu, 1984).

Taylor and Basabe (1984) established correlations between fluoride concentrations in pine needles (Douglas-fir, *Pseudotsuga menziesii*) and annual growth increments, wind pattern, distance from fluoride source (aluminium smelter) and hydrogen fluoride concentrations in emissions. The authors noted that visible fluoride symptoms and over 40% growth reduction occurred in trees that were accumulating fluorides below established “injury threshold levels.” They suggested that synergism between hydrogen fluoride and sulfur dioxide may have given rise to these reduced threshold levels. Vike and Hanjorg (1995) recorded fluoride content and leaf injury in a variety of plant species growing in the vicinity of aluminium smelters in Norway.

Vike (1999) reported that leaf injury appeared at leaf fluoride content levels as low as 30 mg/kg, and damage was restricted to within 2 km of the emission sources. Regression analysis showed a positive correlation between leaf injury and fluoride content of leaves within a locality, but great variation between localities. The author concluded that the establishment of tolerable emission levels must take into account
local dispersal patterns and climatic conditions. Ivinskis and Murray (1984) found that reductions in photosynthetic capacity, chlorophyll \(a\) and \(b\) and leaf area of grey gum (\textit{Eucalyptus punctata}) were all significantly correlated with leaf fluoride content, fluoride in air and distance from an aluminum smelter.

**Chemistry, Physiology and Biochemistry**

1. **Chemistry**

Fluorine is first isolated by Moissan in 1886. Fluorine is estimated to be the 18\textsuperscript{th} most abundant element in the earth's crust. It is the most highly reactive element of the so-called Halogen family. Fluorine is the highest member of Group 17 (VIIA) of the periodic table. This group, the halogens, also includes chloride, bromine, and iodine. As with the other halogens, fluorine occurs as a diatomic molecule, \(F_2\) in its elemental form. Fluorine has relative atomic mass 19.00, exists as the diatomic gas, \(F_2\). Fluorine is the most reactive of all the elements, which may be attributed to its large electro negativity (estimated standard potential +2.85 V). It reacts at room temperature or elevated temperatures with all elements other than nitrogen, oxygen and the lighter noble gases. Fluorine is also notable for its small size; large numbers of fluorine atoms fit around atoms of another element. Important physical and chemical properties of fluorine are MW 37.997, Physical state is gas, Molecular formula is \(F_2\), Melting point is -219.61° C, Boiling point is -188.13° C, and solubility in water is 1.69 mg/L. For hydrogen fluoride the physical and chemical properties viz., MW- 20.006, Physical state- Gas, Molecular formula – \(FH\), Melting point - 83.86° C, Boiling point - 19.51b, Solubility in water is miscible and in organic solvents Benzene (2.54); toluene (1.80); ethanol (very soluble); m-xylene (1.28); tetraline (0.27)b. In sodium fluoride the physical and chemical properties viz., MW- 42.00, Physical state- Cubic or tetragonal crystals, Molecular formula – \(FNa\), Melting point - 993° C, Boiling point – 1.704, Solubility in water is 43g/L at 25° and in organic solvents very slightly soluble in ethanol.

Fluoride is capable of forming compounds with all the elements except helium and neon (Greenwood and Earnshaw, 1984). Fluoride is used as an oxidizer in liquid rocket fuels, in the manufacture of \(UF_6\) for separating the isotopes of Uranium and in the manufacture of hydrogen fluoride, aluminum fluoride, silicon fluorides and especially fluorinated hydrocarbons. It is also used as a constituent of lubricants.
resistant to heating and oxidation, in hydraulic fluids, coatings, fire extinguishers, electrochemical materials, in non-toxic refrigerants (Freons), in insecticides and fungicides. In addition it is used extensively in the production of artificial vessels and heart valves in medicine.

Fluorine reinforces the action of many chemical molecules and this aspect has made this element useful in pharmaceutical industries. The efficacy of a drug frequently depends on the how soon the body metabolizes the molecule and terminates its action. By inserting fluoride at the weak point in the structure of a drug, chemists have made certain pharmaceuticals more resistant to breakdown in the body, thereby reinforcing their action. Some of the most popular fluoride-containing medications are: fluorosteriod, fluorouracil, fluoride containing antihistamines, tranquilizers, anesthetics and diuretics. Besides, sodium fluoride, either by itself or in combination with calcium, Vitamin D and estrogen are prescribed for patients of osteoporosis and the treatment continues for 1-2 years with a dose of 50-80 mg of sodium fluoride per day. Sodium fluoride therapy for osteosclerosis is also common. For dental caries (cavity formation) fluoride tablets, fluoride mouth rinse, fluoride varnish and fluoride containing tooth pastes are also prescribed and used. Fluoride compounds have become extremely important. Fluoride compounds can be classified broadly into the following categories:

1. **Inorganic fluoride compounds**
   a) Complex as with heavy metal ions
      
      Eg. FeF$_6^{3-}$, AlF$_5^-$, MnF$_5^{2-}$, MnF$_3^-$, ZrF$_5^{2-}$, and ThF$_6^{2-}$
      
      These compounds react with vigorously with most organic compounds.
   b) Complexes with non-metallic elements
      
      Eg. Fluoride monoxide, silicon tetrafluoride, Sulfur hexafluoride.
   c) NaF and Alkalifluorides (MHF$_2$)
      
      (where M is the alkali metal)
      
      These compounds are used in fluxes and has been proposed for the removal of hydrogen fluoride from exhaust gases for fluoridation of drinking water.
   d) Industrial compounds
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Eg. Fluorospar (CaF₂) - These compounds are fluorine contain minerals.

Cryolite (3 NaF X AlF₃) – Raw material in the aluminum industry.

Fluorapatite [CaF₂ X 3Ca₃(PO₄)₂] - Consumed in the production of elemental phosphorous, phosphoric acid and phosphate fertilizers.

Eg. Silicon tetrafluoride (SiF₄) – Manufacture of super phosphate fertilizers, elemental phosphorous, wet process phosphoric acid, aluminum and brick and tile products Hexafluorosilicate ion (SiF₆²⁻) - It is very toxic Sodium fluorosilicate – Used to fluoridate drinking water. Fluorosilicic acid – Suitable for dry dosage fluoridation equipment.

f) Synthetic fluoride

Eg. Sodium monofluorophosphate (Na₂FPO₃) - Used in the fluoride dentrifice industry.

2. Organic fluoride compounds

1. Fluorocarbons (Saturated and unsaturated compounds of carbon, hydrogen, fluorine and other halogens). - Used aerosol propellants, refrigerants, solvents and blowing agents.

2. Organo fluorine anaesthetics eg. Methoxy-fluorine (CH₃-O-CF₂-CCl₂H), Enflurane (CHF₂-O-CF₂-CCl) and isoflurane (CHF₂-O-CHCl-CF₃) - Used in anaesthetics.

3. Natural organic fluorides – eg. fluoro acetic acid, fluoro-oleic acid and fluorocitrate.

These have reported to occur in over 20 tropical or arid-zone plants and are poisonous to animals.

2. Physiology and Biochemistry

High levels of F⁻ are toxic (Fluorosis) F⁻ affects several enzymes. Excess F⁻ decreases fatty acid oxidase in rat kidney, and partially inhibits intestinal lipase. Fatty acid utilization is generally impaired in fluorosis. Carbohydrate metabolism is also affected, probably due to inhibition of enolase and a shift of the NAD/NADH ratio in favour of NADH. The role of F⁻ has been clearly demonstrated in humans. F⁻ is an anion found in bone and tooth appatite. Small quantities are beneficial in lowering
the incidence of caries, and this cariostratic effect of $F^-$ has been clearly demonstrated in humans. The role of $F^-$ in the inhibition of osteoporosis is less certain.

Skeletal fluorosis is the most common disease in endemic areas with soils and water containing high fluoride concentration. In non-endemic areas, skeletal fluorosis occurs as a result of industrial exposure. Skeletal fluorosis was first reported in Copenhagen, during a routine examination of cryolite workers (Moller and Gudjonsson, 1933). The disease was described in detail by Roholm (1937). He observed the following skeletal manifestations: increase in bone density, uneven of bone contours and trabeculae, thickening of bones, irregular periosteal growth and calcification in ligaments, tendon and muscle insertations with the increased radiological density. Clinical signs and symptoms may become more severe, especially pain in joints of hands, feet, knees and spine with increasing severity; the pain increases and the movement of the vertebral column and lower limbs becomes limited.

Corresponding observations were reported by many researchers in many countries. Singh and Jolly (1970) reported that crippling fluorosis in human beings was the result of continuous daily intake of 20-80 mg fluoride for 10-20 years. Relatively marked osteofluorotic manifestations were connected with fluoride levels as low as 1-3 mg/liter of drinking water. Singh et al., (1961b), Jolly et al., (1969); and Jolly (1976) indicated that a daily intake exceeding 8 mg in adults would be detrimental. Poor nutrition, including calcium deficiency and hard manual labour appears to play an additional role in tropical areas with endemic fluorosis (Siddiqi, 1955 and Singh et al., 1961a). In temperate countries, manifestations of skeletal fluorosis have not been detected in relation to drinking-water containing fluoride levels below 4 mg/liter (Victoria committee, 1980). Christie (1980) found crippling deformities among the children who consume, the drinking water containing 21 mg/liter of fluoride. He also observed several abnormalities on radiographic examination, including increased activity and height of the posterior ribs, increased anteroposterior diameter of the chest, vertebral bodies with increased width and decreased height, considerable exaggeration of the normal shape of pelvis, joint deformities and lateral bowing of the femur. "Genu valgum" in children with life-
long exposure were reported from India (Teotia et al., 1971; Krishnamachari and Krishnaswamy, 1973).

Non Skeletal manifestations are associated with acute poisoning. The manifestations include nervousness, depression, tingling sensation of fingers/toes, excessive thirst and tendency to urinate frequently and excessively, muscle weakness, stiffness, muscle spasm and pain, nausea and vomiting; and allergic manifestations can be of various natures, very painful skin rashes which are peri vascular inflammations. Hodge and Smith (1981) grouped most of the acute fluoride effects into 4 categories of major functional derangements 1. Enzyme inhibition 2. Calcium complex formation 3. shock and 4. specific organ injury.

1. Enzyme inhibition: Fluoride particularly inhibits metallo enzymes involved in carrying vital functions such as the initiation and transmission of nerve impulses. The fluoride ions may exert a direct action on enzymes but, more frequently, the effect is indirect by complexing with metals of enzymes. Low concentrations of fluoride in serum stabilize and activate several isolated as well as membrane-bound enzyme systems. But higher concentrations of fluoride in serum inhibit many enzymes (Hodge and Smith, 1965 a and b; Taves, 1970 ; Wiseman, 1970; USEPA, 1980 and SOU 1981).


3. Shock: Features like drop in blood pressure, increasing in rate and depth of respiration followed by respiratory failure have been described in man and dogs (National Academy of Sciences, 1971 and Messer, 1984). Baltazar et al., (1980) observed Hyperkalemia with electro-cardio graphic changes in man and dogs.

Fluoride is known to interact with body tissues in many ways. Fluoride accumulates in almost all the body tissues. Carlson et al., (1960) observed that solutions of fluoride salts are rapidly and almost completely absorbed from the
gastrointestinal tract, probably by simple diffusion. Dinman et al., (1976) observed the respiratory tract is the major route of absorption of both gaseous and particulate fluoride. Hydrogen fluoride being highly soluble in water is rapidly taken up by the upper respiratory tract. On absorption of fluoride, it accumulates in almost all the body tissues. Approximately 99 percent of the fluoride in the body is accumulated in the skeleton. The rest is distributed between the blood and soft tissues. Susheela et al., (1982) reported localization of highest amount of fluoride in cancellous bone (spongy bone) than other cortical variety, possible due to its greater surface area exposed to circulation. Fluoride can depress the intracellular metabolism (Baudene, 1975). Fluoride has been shown to inhibit a variety of enzymes at concentrations ranging from $10^{-7}$ to $10^{-1}$ M (Wiseman, 1970). It has effect on enzymes associated with carbohydrate metabolism (Wiseman, 1970; McGown and Suttie, 1977; Dost et al., 1977 and Miller et al., 1978) and protein synthesis (Hoerz and McCarty, 1971; Godchaux and Atwood, 1976 and Holland, 1980).

Humans

Excess fluoride in water or air can cause several health problems, since all fluoride compounds are poisonous. An individual may suffer either from non-skeletal manifestations or skeletal fluorosis or dental fluorosis or a combination of these manifestations. Excessive ingestion of fluoride during the period of tooth development may result in defective tooth formation which is called dental fluorosis. It is a disturbance affecting the enamel during formation, hence all damage occurs before the eruption of the tooth. The brown-black discoloration is, however, a secondary phenomenon due to the deposition of stains from the oral cavity onto the spongy surface of severely mottled areas. Dean (1942) observed dental fluorosis at different levels of fluoride in the drinking water. The enamel-forming cells and the ameloblasts are affected by fluoride. The maturation of the enamel is delayed, and the general mineralization process may be inhibited, perhaps through interference with nucleation and crystal growth. Calcium homeostatic mechanisms may be affected. In severe dental fluorosis histological changes are found in enamel and dentin (Fejerskov et al., 1979).
Vertebrates

Various items of food have been analyzed and fluoride-content has been studied by researchers. Tea leaves accumulate more fluoride than any other edible plant. According to Srebrink and Mijnsbrugge (1976) fifteen different kinds of dry tea leaves contains 50 to 125 ppm fluoride. Drinking tea is a good source of fluoride which provides 0.048-0.97 mg/day (Kondo et al., 1973; Ulvstad, 1973). Among food derived from animal sources seafood and fish are richest in fluoride (Neilson and Sandstead, 1974). The calcium in fish absorbs fluoride from sea water, especially to their outer portions and to their bones. The sea fish, Mackeral contains 27 ppm (fresh weight) and dog-fish protein contains 761 ppm. Wild animals such as deer and rabbits also accumulate fluoride in their bones, especially in industrial areas, but predators generally demonstrate higher levels than their prey (Kay, 1975).

The fluoride concentration is more in cooked food compared to un-cooked food. It is common knowledge that Indian food is highly spiced, and the commonly used spices have a very high fluoride concentration. Although the rural Indian population can ill afford to use expensive spices, they invariably consume a huge quantity of fluoride in the form of chewing either the areca nut betel leaf or tobacco, which have higher fluoride content. As there is movement of food grains and other agricultural products among different states within the country, there is considerable possibility that even in non-endemic states health problems exist due to excess ingestion of fluorides. Fluoride, when in optimal quantities, is a vital necessity for man and animals. Fluorine is a component of bones and teeth. Minimal quantity of fluoride has been shown to benefit man by producing an increased resistance to dental caries. People using water with fluoride content of 1 mg/liter or more were found to have about 50 percent less dental caries than those with a supply containing 0.1-0.3 mg fluoride per liter (Dean et al., 1949 and 1941 b). The studies of Murray and Rugg-gumm (1979) showed a reduction in caries in the average of 50-75 percent for permanent teeth and about 50 percent for primary teeth in children, 5-15 years of age, following daily intake of fluorinated water. Tooth tissues contain about 0.02 percent of fluoride, its major part being in tooth enamel whose composition is close to formula $\text{Ca}_5\text{F} (\text{PO}_4)_3$. According to Messer (1984), fluoride promotes the initial deposition of apatitic mineral in bones and teeth which involves conversion of transitional forms of calcium phosphate to the crystalline apatite.
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Mammals

The original findings of fluoride effects on mammals were from studies in the field on domestic animals such as sheep (Moul, 1944; Harvey, 1952; Peirce, 1952) and cattle (Rand and Schmidt, 1952; Neeley and Harbaugh, 1954; Burns and Allcroft, 1964; Allcroft and Burns, 1968; Krook and Maylin, 1979). Investigations of the effects of fluoride on wildlife have focused on impacts on the structural integrity of teeth and bone. Most observations have involved large herbivores. For example, dental and skeletal lesions were found in mule deer (*Odocoileus hemionus*), elk (*Cervus canadensis*) and American bison (*Bison bison*) exposed to elevated levels (no specific anthropogenic sources identified) of fluoride in Utah, Idaho, Montana and Wyoming, USA (Shupe et al., 1984). Fluoride levels in ribs ranged from 2800 to 6800 mg/kg on a fat-free basis, compared with control deer, which had levels ranging from 160 to 460 mg/kg (Newman and Yu, 1976). Karstad (1967) found that mandibular bone fluoride contents of 4300–7125 mg/kg in mule deer (*Odocoileus hemionus*) near an industrial complex were associated with pitting and black discoloration of teeth, abnormal tooth wear and fractures of teeth and jaw bones. Kierdorf et al., (1996) monitored mandibular bone fluoride concentrations and frequency and intensity of fluoride-induced dental lesions in red deer in the Czech Republic and Germany. Kierdorf et al. (1997) concluded that increased fluoride exposure of deer leads to reduced mineral content and mineral density of antler bone and that it is the rapidity of their growth and mineralization that makes antlers especially susceptible to fluoride action.

Cattle (Ungulates)

Most of the early work on mammals was carried out on domesticated ungulates (Suttie, 1983). Fluorosis has been observed in experimental studies on cattle and sheep exposed to fluoride. Cattle are less tolerant of fluoride toxicity than are other livestock (Phillips and Suttie, 1960). In long-term experiments with beef cattle, 30 mg/kg of dietary fluoride caused excessive wear and staining of teeth (Hobbs and Merriman, 1959). Suttie et al. (1957) found that lactating cows could tolerate 30 mg/kg, with a rate of 50 mg/kg causing fluorosis within 3–5 years. Tolerance levels have been identified for domesticated animals based on clinical signs and lesions. Tolerance levels in feed range from 30–40 mg/kg dry weight in dairy
cattle to 150 mg/kg in lambs; in water, 0 mg/litre in dairy cattle to 12–15 mg/litre in lambs (Shupe and Oison, 1983; Cronin et al., 2000).

**Wild animals**

Deer mice (*Peromyscus maniculatus*) fed diets of 38 (control), 1065, 1355 or 1936 mg fluoride/kg diet dry weight (as sodium fluoride) for 8 weeks exhibited, at all concentrations above the control, marked weight loss, mortality, changes in femur size and dental disfigurement (Newman and Markey, 1976). Fluoride was suggested as the cause of reduced milk production with subsequent mortality of kits in farm-raised red foxes (*Vulpes vulpes*) fed a diet containing 98–137 mg fluoride/kg dry weight (Eckerlin et al., 1986). Aulerich et al. (1987) fed mink (*Mustela vison*) diets containing 35–385 mg fluoride/kg for 382 days. No significant differences were observed in body weight gains or fur quality between dosed and control mink. No adverse effects of fluoride on breeding, gestation, whelping or lactation were found. Survival was adversely affected at 385 mg fluoride/kg; some of the surviving mink at this concentration had weakened frontal, parietal and femoral bones. Chemical analyses for fluoride generally reflected the levels ingested. After data were evaluated, a tolerance level in the feed of 50 mg fluoride/kg for breeding stock was recommended (Shupe et al., 1987).

**Aquatic Animals**

Water borne fluoride has been said to represent the largest single source of daily intake of this element. Fluorides are almost found in all water supplies in varying concentrations (Maier, 1963). The natural content of fluoride in water from different areas depends on such factors as the geological, physical and chemical characteristics, the consistency of the soil, the porosity of rocks, the pH and temperature, the complexing action of other elements and the depth of wells (Worl et al., 1973). Water usually found at the foot mountains and in areas with geological deposits of marine origin have high F content. Typical examples are the geographical belt from Syria through Jordan, Egypt, the Libiyan Arab Jamhiria and Algeria to Morocco and the Rift valley through Sudan and Kenya. Another belt is the one stretching from Turkey to India, Northern Thailand and China. Such areas can be found in America and Japan. Seawater contains fluoride at levels of 0.8 to 1.4 mg/l whereas in freshwater, it is generally below 0.5 mg/l even though the concentration as
high as 95 mg/l is recorded in some parts (Tanganyika, 1955). The highest fluoride content with 2800 mg/l was found in lake Nakuru in the Rift valley in Kenya. The soil at the lake Nakuru Shore contained up to 5600 mg F/kg and the dust in the huts of the local inhabitants contained 150 mg/kg (Williamson, 1953). America has water with high fluoride of 25 ppm.

Short-term fluoride toxicity data are available for a number of invertebrate species, the majority of them marine varieties. Water fleas are killed or immobilized by concentrations of various fluoride compounds ranging from 5 to 500 ppm (Sanders and O.B. Cope, 1966; Anderson, 1946; Bringmann and R. Kuhn., 1959). Lobsters are not harmed by 5 ppm fluoride (Stewart and Cornick., 1964). Mussels may be killed by 1.4 to 7.2 ppm (Hemens and Warwic., 1972) and concentrations of 20 ppm or higher for extended periods have been shown to be toxic or lethal to oysters, two species of crabs, and a sand shrimp, but not to two types of prawn. More significant than the lethal effects of high concentrations of from 100 to 300 ppm ((Moore, 1971). The entry of fluoride into food chains through bioconcentration in aquatic invertebrates is a subject in need of much more careful research. Studies of the effects of fluoride on fish are far more numerous than for any other form of aquatic life (Neuhold and Sigler., 1960). Short-term lethal effects may occur at concentrations as low as 3 ppm in sensitive species (for example, rainbow trout), while other fish are not damaged until fluoride levels reach 100 ppm. Water temperature, hardness, chlorinity and other environmental factors, as well as the age and physiological state of the fish, can influence the toxicity of a given concentration of fluoride (Sigler and Newhold, 1972).

Sub lethal concentrations may have adverse effects on fish behavior or reproduction, which could be ecologically significant. Research findings are few and not confirmed, but trout eggs seem to be delayed in development and hatching by 1.5 ppm fluoride (Ellis et al. 1948). Fish are important food-chain organisms, and the ability of many fish, like many other vertebrates, to accumulate elevated fluoride levels in their skeletons (Fisher and Prival, 1973) can introduce the contaminant into the diet of fish-eating predator. Levels of 550 to 6,800 ppm have been reported in bones of ocean fish, and 400 to 1,600 ppm in trout from a naturally high-fluoride
stream in Yellowstone National Park. Such accumulation might pose a hazard to animals that eat whole fish.

Data on other aquatic vertebrates which may be exposed to fluoride are sparse. Frogs were killed in one week by 900 ppm fluoride and decreased red and white blood cell counts were observed in frogs kept in fluoride concentrations of 5 to 300 ppm (Kaplan et al., 1964). There have also been indications that sub lethal fluoride concentrations may adversely affect amphibian reproductive cycles (Cameron, 1940). Frog eggs were retarded in development but hatched prematurely in 1 ppm fluoride in well water, higher concentrations (13 to 450 ppm) had the same effects on toad eggs, and metamorphosis in tadpoles was significantly delayed by fluoride at 0.5 and 4.5 ppm, (Kuusisto and Telkka, 1961).

Most research on the effects of fluoride on aquatic organisms dates back to the early 1960s or before, and more definitive studies are required on the potential hazards suggested here. There is also a pressing need to examine the potential impact of chronic, low-level bioaccumulation of fluoride on predatory animals higher in aquatic based food chains. As is the case with fluoride air pollution, the logic of ecosystem, energy; and nutrient flow patterns suggests that species at the highest levels of a food chain are likely to bear the greatest risk of harm but virtually no effort has been made to look for such damage. If fluoride has had such adverse effects on aquatic wildlife, they have thus far been too subtle to attract attention, in the absence of any substantive research data; it would be unwise to assume that no risks exist.

Aquatic organisms

Kudo and Garrec (1983) simulated the accidental release of ammonium fluoride into a pond. Mean fluoride concentrations increased from 0.2 to 7.3 mg/litre following the release. Fluoride levels returned to background concentrations within 30 days. No visible toxic effects on plants, algae, molluscs or fish were observed during the 30-day period. No details regarding the chemical characteristics of the pond water were given. Ares et al. (1983) monitored fluoride concentrations and diatom populations in seawater near an aluminum smelter in southern Argentina. Fluoride concentrations primarily ranged from 1.1 to 1.3 mg/litre. Anthropogenic emissions accounted for 6–8% of the variance of fluoride levels in the waters. The correlations
observed between fluoride concentrations and some structural characteristics of the diatom community did not show significant effects of the discharged fluoride. Fluoride-loaded effluent adversely affected the species richness of a marine encrusting community for up to 400 m from the point of discharge. Fluoride concentrations greater than 50 mg/litre (the concentration of fluoride in effluent at which mortality was observed in the laboratory) seldom extended for more than 20 m from the outfall. The authors concluded that a sub lethal effect of fluoride and/or some other effluent component appeared to be producing the observed distribution (Pankhurst et al., 1980).

High mortality and delayed migration were observed in Pacific salmon (Oncorhynchus sp.) migrating upstream near an aluminum plant on the Columbia River, USA (Washington/Oregon border). Fluoride levels in the water ranged between 0.3 and 0.5 mg/liter during 1982. Bioassay experiments on adult salmon suggested that fluoride concentrations of 0.5 mg/litre would adversely affect migration. Between 1983 and 1986, discharges from the aluminum plant were reduced, there was a corresponding drop in fluoride concentrations, and fish mortality and migration delays were decreased (Damkaer and Dey, 1989).

Amphibians

Mishra and Mohapatra (1998) monitored toads (Bufo melanosticus) at a fluoride-contaminated site at Hirakud, India. Mean haemoglobin content, total red blood cell count and haematocrit in blood samples were found to be significantly reduced, whereas mean corpuscular concentration and volume were significantly elevated when compared with toads at an uncontaminated site. Mean bone fluoride concentrations were 2736 mg/kg at the contaminated site and 241 mg/kg at the control site.

Fishes

The acute toxicity of fluoride to fish is summarized in Table 16. Ninety-six-hour LC50s for freshwater fish range from 51 mg fluoride/litre (rainbow trout, Oncorhynchus mykiss) to 460 mg fluoride/litre (threespine stickleback, Gasterosteus aculeatus). All of the acute toxicity tests (96 h) on marine fish gave results greater than 100 mg fluoride/litre. Inorganic fluoride toxicity to freshwater fish appears to be
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negatively correlated with water hardness (due to the complexation of fluoride ions with calcium) and positively correlated with temperature (Angelovic et al., 1961; Pimentel and Bulkley, 1983; Smith et al., 1985). The 96-h LC50 for rainbow trout exposed to sodium fluoride in soft water (17 mg calcium carbonate/litre) was 51 mg fluoride/litre. Increasing the water hardness to 49 mg calcium carbonate/litre doubled the LC50 to 128 mg fluoride/litre; a further increase in water hardness to 182 mg calcium carbonate/litre led to an LC50 of 140 mg fluoride/litre (Pimentel and Bulkley, 1983). Angelovic et al., (1961) found that increasing temperature significantly increased the toxicity of fluoride to rainbow trout at temperatures ranging from 7.2 to 24° C.

Camargo and Tarazona (1991) exposed rainbow trout (O. mykiss) and brown trout (Salmo trutta) to fluoride in soft water (22 mg calcium carbonate/litre) during short-term toxicity tests. For rainbow trout, 120-, 144-, 168- and 192-h LC50s were 92.4, 85.1, 73.4 and 64.1 mg fluoride/litre, respectively; for brown trout, they were 135.6, 118.5, 105.1 and 97.5 mg fluoride/litre, respectively. The symptoms of acute fluoride intoxication included lethargy, violent and erratic movement and death. The authors postulated that the variation in the response of fish to fluoride could be due to a chloride–fluoride excretion mechanism over the epithelial tissues (Sigler and Neuhold, 1972). The eggs of the freshwater catla (Catla catla) were exposed to fluoride concentrations ranging from 1.9 to >16 mg/litre from both effluent and sodium fluoride dilutions. Hatching occurred after 6 h in both controls and those exposed to 1.9 mg fluoride/litre. At concentrations of $3.2 mg fluoride/litre for effluent dilutions and $3.6 mg fluoride/litre for sodium fluoride dilutions, hatching was delayed by 1–2h. The authors stated that the low pH (4.1) of the effluent may have contributed to its toxicity. The weight of eggs exposed to fluoride decreased with increasing fluoride concentration and exposure time. Significant decreases in egg protein were observed at fluoride concentrations causing delayed hatching. The toxicity of fluoride to eggs was more related to the availability of fluoride ions than to total fluoride in the media (Pillai and Mane, 1984).

Amphibians (Frogs)

Kaplan et al. (1964) found little external evidence of toxicity in adult leopard frogs (Rana pipiens) exposed to fluoride concentrations ranging from 5 to 50 mg/litre
for 30 days. Total red and white cell counts were reduced at all fluoride exposure concentrations; however, no statistical analysis was carried out. At fluoride concentrations ranging from 50 to 300 mg/litre, the survival time decreased with increasing exposure concentration. All frogs died within 30 days at both 250 and 300 mg fluoride/litre. Kuusisto and Telkkä (1961) exposed frog *(Rana temporaria)* larvae to sodium fluoride concentrations of 1, 2 and 10 mg/litre.

**Birds**

Guenter and Hahn (1986) fed white leghorn hens on a diet containing up to 1300 mg fluoride/kg for 252 days. Concentrations of $1000 \text{ mg/kg}$ resulted in significant depression of feed intake, body weight gain, feed efficiency and egg quality. In a second experiment, pullets were fed 1300 mg/kg for 49 days; similar results were obtained, but the addition of aluminum (1040 mg/kg) to the diet reduced the effects. Chan *et al.*, (1973) found no effect on growth in Japanese quail *(Coturnix coturnix japonica)* fed diets resulting in average tibial fluoride concentrations of 13–2223 mg/kg ash weight. Bird and Massari (1983) fed kestrels on a diet to which flour contaminated with fluoride (10, 50 or 500 mg/kg) had been applied. The birds receiving the highest fluoride dose died within 6 days. Fluoride at the lower doses had no effect on clutch size, hatchability or fledging success but was associated with a higher fertility. Eggs laid by kestrels at 50 mg/kg had significantly thicker shells. Lower reproductive success of eastern screech-owls *(Otus asio)* was noted when birds were fed 90 mg fluoride/kg diet wet weight (as sodium fluoride), but not when fed 18 mg fluoride/kg diet (Hoffman *et al.*, 1985 and Pattee *et al.*, 1988).

Van Toledo (1978) found that the number of avian species near fluoride-emitting aluminium factories in Europe was depressed. They speculated that the reductions were due to fluoride emissions. Newman (1977) believed that the nesting density of house martins *(Delichon urbica)* was reduced near an aluminum plant with high fluoride emissions. However, there were many other air pollutants present, and so it is difficult to establish a causal relationship with fluoride in particular. Henny and Burke (1990) monitored black-crowned night herons *(Nycticorax nycticorax)* living near a phosphate-processing complex in Idaho, USA. Fluoride in femurs ranged from 540 to 11 000 mg/kg ash weight and increased with age.
Invertebrates

The acute toxicity of fluoride to aquatic invertebrates is summarized in Table 15. Forty-eight-hour LC50s/EC50s range from 53 to 304 mg/litre. Hemens and Warwick (1972) found that fluoride concentrations of up to 100 mg/litre caused no mortality in prawns exposed for 96 h. However, the brown mussel (Perna perna) showed up to 30% mortality at an initial fluoride concentration of 7.2 mg/litre during a 5-day test (the background fluoride concentration was 1 mg/litre). LeBlanc (1980) found a 48-h NOEC for Daphnia magna of 50 mg fluoride/litre, whereas Kühn et al., (1989) reported a no-effect concentration of 231 mg fluoride/litre (24 h). Camargo and La Point (1995) calculated “safe concentrations” (8760-h EC 0.01s) for the last-instar larvae of several net-spinning caddisfly species. “Safe concentrations” ranged from 0.39 mg fluoride/litre (Hydropsyche pellucidula) to 1.18 (Hydropsyche lobata) and 1.79 mg fluoride/litre (Chimarra marginata). Pankhurst et al., (1980) performed toxicity tests on the blue mussel (Mytilus edulis). Nell and Livanos (1988) found that weight gains in Sydney rock oysters (Saccostrea commercialis) decreased linearly (P < 0.01) with increasing fluoride additions from 0 to 30 mg/litre, and there was a 20% growth depression at the highest fluoride concentration.

Fluoride (5.88 mg/litre) had no effect on the survival of mud crabs (Tylodiplax blephariskios) or the survival and growth of penaeid shrimps (Penaeus indicus) during 68-day exposures when compared with controls (0.89 mg fluoride/litre). In a 113-day test, shrimps gained significantly more weight during exposure to fluoride (5.5mg/litre) than did controls. The authors attributed this to variations in the food source within the tanks (Hemens et al., 1975). The fingernail clam (Musculium transversum) appears to be one of the most sensitive freshwater species tested. Sparks et al. (1983) conducted an 8-week flow-through experiment in which statistically significant mortality (50%) was observed at a concentration of 2.8 mg fluoride/litre. A maximum acceptable toxicant concentration (MATC) was set at between 5.0 and 6.2 mg fluoride/litre. However, female fecundity appeared to be the most sensitive parameter, and a mean MATC based on this parameter was 4.2 mg fluoride/litre (Connell and Airey, 1982).

In a 3-week exposure test, survival and reproduction of Daphnia magna were studied (Fieser et al., 1986). Impairment of reproduction was observed at fluoride
concentrations greater than 26 mg/litre. A concentration of 35 mg fluoride/litre reduced the neonate production to 44% of controls and the average number of live young dropped by more than 98% at 49 mg fluoride/litre. However, no statistics were performed in the study. Dave (1984) calculated that the NOEC for growth in *Daphnia magna* after 7 and 21 days was between 3.7 and 7.4 mg fluoride/litre. Similarly, for parthenogenesis reproduction, the 21-day NOEC was between 3.7 and 7.4 mg fluoride/litre. The “safe” concentration, equivalent to the geometric mean of the NOEC or MATC in hard water, was 4.4 mg fluoride/litre. Kühn *et al.* (1989) reported a 21-day NOEC of 14 mg fluoride/litre for *Daphnia magna*; the most sensitive parameter was reproduction rate. Johansson and Johansson (1972) found that egg production and survival were adversely affected in flour beetles (*Tribolium confusum*) exposed to flour containing 4524 mg fluoride/kg (0.1% sodium fluoride) for up to 27 days. However, short-term (1–7 days) exposure to fluoride concentrations of 452.4 mg/kg (0.01% sodium fluoride) produced significant stimulation of egg production.

Hughes *et al.* (1985) fed cabbage looper (*Trichoplusia ni*) larvae on a wheat germ diet dosed with sodium fluoride (50 and 187 mg fluoride/kg dry weight) or potassium fluoride (48 and 235 mg fluoride/kg dry weight) or a diet of hydrogen fluoride-fumigated leaves (40–187 mg fluoride/kg dry weight). Larval feeding, growth and rate of development were generally reduced on diets containing sodium fluoride and potassium fluoride. The same parameters were generally greater with or unaffected by diets containing fumigated leaves. Wang and Bian (1988) exposed silkworms (*Bombyx mori*) to mulberry (*Morus alba*) leaves containing fluoride concentrations ranging from 10 to 200 mg/kg. The threshold concentration for mortality was 30 mg/kg; 30% mortality was observed at concentrations of 30–50 mg fluoride/kg, 70% at 50–120 mg fluoride/kg and 95% at 120–200 mg fluoride/kg. There was a close correlation between cocoon development and the fluoride content of leaves, with >80 mg fluoride/kg severely inhibiting cocoon production. Survival of isopods (woodlouse, *Porcellio scaber*) was not affected after 4 weeks of exposure to fluoride levels in litter up to 3230 mg/kg (170 μmol/g) (Van Wensem and Adema, 1991).
Davies et al., (1998) found no effect on the reproduction of aphids (Aphis fabae) feeding on bean (Vicia faba) plants (12 days) that had been previously exposed to either sodium fluoride in nutrient solution (15 μg fluoride/cm³) or hydrogen fluoride via fumigation (6.5 μg fluoride/m³). Plants accumulated more fluoride in the shoots during fumigation and at levels of up to 200 mg fluoride/kg; aphids accumulated up to, 300 mg fluoride/kg from these plants. Similarly, Port et al., (1998) found no effect of hydrogen fluoride on the growth and survival of cabbage white caterpillars (Pieris brassicae) at a dietary concentration of 178.3 mg fluoride/kg.

Insects

Mayer et al., (1988) conducted a 3-year study on honeybees (Apis mellifera) in Puyallup Valley, Washington, USA, near an aluminum plant. The mean fluoride content of bees from a site 0.8 km downwind of the plant ranged from 82 to 261 mg/kg dry weight. No significant effect of fluoride on brood survival, brood development or honey production was found. Fluorotic silkworm showed body color and behavior changes and the data revealed that there was obvious dose effect and times relation ship with the pathological changes and gene expressions (Hong Zhou et al., 2008). Expression analysis indicated that Bmcyp306a1 was exclusively; expressed in 441 DZ and 441 F (resistant strains) and was down regulated under fluoride treatment and the expression profiles of Bycp306a1 suggested that p 450 gene was crucial to physiological modification and might be involved in fluoride resistance (Hong Zhou et al., 2008). According to Zhao et al., (2007) it is important to the farmers in Jiangsu, Zhejiang, Anhui, Sichuan, Shandong and other provinces to breeding a new silkworm varieties that are fluoride-tolerant, disease-resistant, adversity-resistant and hyper silkgeneous for rearing in both spring and autumn seasons. In the filed observations at polluted sites Pyris brassicae absorbed little fluoride by surface contamination (Port et al., 1998). Some silkworm varieties with special economic characters are for special purposes were bred for example fluoride pollution has been seriously disturbed commercial cocoon production due to the rapid development of rural industry, since early 80s of 20th century in China (FAO). In order to stabilize cocoon production in the major sericultural region, some highly fluoride tolerate varieties were bred. Ituafen GW x Hue. (Chen et al., 2002) can
normally developed when fluoride content reached 60 mg/kg in the mulberry leaves during 1\textsuperscript{st} – 3\textsuperscript{rd} instar and 120 mg/kg during 4\textsuperscript{th} – 5\textsuperscript{th} instar.

The LC\textsubscript{50} of fluoride in mulberry leaves to silkworms was investigated at 4\textsuperscript{th} instar stage for 50 varieties of silkworm \textit{Bombyx mori}. from three localities in China and found large variations in fluoride tolerance among the silkworm varieties which ranged from 19 to 693 mgF/kg of fried mulberry leaves for the LC\textsubscript{50} (Yuin Chen, 2003). Thus there was considerable difference in the LC\textsubscript{50} for the same variety of silkworm maintained in different localities. Thereby indicate adapting to atmospheric pollution. Ants decreased in abundance above low fluoride levels (<1.85 \textmu mol.g\textsuperscript{-1}) the sensitivity of fluoride demonstration by ants may make them particularly good candidates as bio indicator of fluoride stress (Madden and Fox, 1997).

Among the four major insect groups such as pollinators, predators, foliage feeders and cambium region feeders 100\% high fluoride levels was observed in predatory insects which indicates fluorides are either accumulated by respiration, or are posted along the food chain (Dewey and Jerold, 1973). At any given site, amounts of environmental fluoride were determined partly by distance and direction from the pot-rooms of the plant (a function of prevailing winds), modified by the presence of trees which acted as collectors for a gaseous and particulate fluoride (Walton, 1986). The overall sequence of increasing fluoride accumulation in invertebrate groups was; herbivores-carnivores-predator-scavengers (Alan and Buse, 1986). Jorgen and Johannes (1991) observed fluoride accumulation in different earthworm species around industrial works like aluminum smelters, steel works and coal fire power plants. The deleterious effects of fluoride on silkworms have been well documented in China, Japan and India (Fuji and Honda, 1972; Kuribayashi, 1977; 1985; Wu \textit{et al.}, 1998; Chen, 1993; Chen and Wu, 1994; Aftab and Chandrakala, 1999; Aftab \textit{et al.}, 1999, Farhana Begum, 2003, Anilkumar, 2006 and Vidyunjmala \textit{et al.}, 2009).

Field studies of a wide variety of invertebrate taxa invariably indicate that samples taken from areas near fluoride pollution sources have greatly; elevated fluoride loads (Garrec and Plebin, 1984; Walton, 1986). The economic loss to silkworm producers during those two seasons was estimated about US $ 1.21 and 1.57 million, respectively (Ma and Xu 1988). There is much evidence that the widely
distributed brick kilns were the main fluoride pollution source (Tong, 1988). Hosagoudar et al., (2004) observed that fluoride apart from exhibition harmful effects on silkworm growth and development, however, it low concentration do play a vital role in inducing some positive effect on fecundity and cocoon traits.

However, fluoride is not studied extensively for its toxicological potential in economic silkworm and only a very few reports are available on the toxicity of fluoride. But the available is not sufficient enough to arrive at a definitive conclusion to say fluoride is toxic or non-toxic in silkworm. Hence, the present investigation was carried out to evaluate its toxic potential using a number of test systems.

OBJECTIVES OF THE PRESENT INVESTIGATION

Mulberry being a foliage crop is grown extensively in the states of Andhra Pradesh, Karnataka and Tamil Nadu, for feeding of silkworms. The mulberry gardens in these regions occasionally get contaminated with many kinds of pollutants and problems have cropped up in sericulture due to intoxication of silkworms fed on contaminated mulberry leaves. The quantity and quality of mulberry leaf is now the concern of experts in all silk producing countries of the world. The quality of mulberry leaf depends on the location of the source and the state of environmental factors such as soil, water and air. It is important to note that generally sericulture is being practiced in drought prone areas of all three states where the soils and ground waters are occasionally contaminated with fluoride ranging 2- 2.5 ppm. Of the different environmental factors, fluoride exerts a profound influence on the growth of the silkworms especially in drought prone areas of India. Fluoride generally regarded as highly toxic to all organisms. Similarly, there also exists of its influence on the cocoon yield. In commercial production of mulberry cocoons, it is aimed at optimum environmental requirements which are essential for maximum productivity of good quality cocoons of very high silk content. Higher concentrations of fluoride acts as a cumulative poison and cause adverse effects on mulberry silkworm larvae at physiological and economic aspects in India and in China (Aftab et al., 1992; Chen et al., 2005; Vidyummala, et al., 2009). But, no comprehensive, comparative and sequential studies are made earlier on the physical, physiological, biochemical and histological factors of silkworm exposed to toxicity of fluoride at different doses and periods of exposure.
Chapter 1: General Introduction

Hence, an attempt is made in this investigation to study the effect of fluoride on various aspects of important physical, physiological, biochemical and histological parameters such as food budget, toxicity evaluation, bioaccumulation, histopathological studies in different organs of silkworms, some aspects of metabolisms of carbohydrate, protein, enzymatic studies at different fluoride doses and durations. As silkworm is a voracious feeder of mulberry leaves, a considerable, rapid growth of about ten thousand times from hatching to pupation within a span of 25-30 days is noticed, especially in 5th instar. Therefore the active V instar silkworm is selected as experimental animals in this investigation, instead I, II, III and IV instar silkworms which are very sensitive to any toxicant even at lower dosage. Further, the ambient temperature such as optimum temperature 25 °C ± 1 and relative humidity 60-70% was selected. Added to this, most of the studies are confined to the other insects and different races and combinations of silkworms to fluoride toxicity at a random doses and durations, but the study of the active instar of the silkworm larvae at sub lethal and sub-sub lethal doses on morphological, biochemical and histological aspects in the PM x CSR₂ cross breed silkworm exposed to fluoride is meager. This wide lacuna in the filed of insect toxicology, respect of silkworm prompted to select this investigation.

PM x CSR₂ silkworm combination is commercially important and well acclimatized to the environment of southern peninsula and reared by 70% of sericulturists of South India, this cross breed silkworm combination especially the V instar exposed to sub lethal and sub-sub lethal doses of fluoride at different periods of exposure is selected in this study, as a matter of field applicability. This also helps in determining the safe periods or safe sub lethal or sub-sub lethal periods for various instars of silkworms and the results of this project give scope to know indirectly the economic values for the maintenance of mulberry and cocoon production under normal and fluoride pollution conditions. Further, the work can lead to identify the fluoride toxicity alleviating mechanisms/substances in future.