

ALGAL AND CYANOBACTERIAL RESPONSES TO FLUORIDE

Monica Bhatnagar^a and Ashish Bhatnagar
Ajmer (Rajasthan), India

SUMMARY: This review deals with the distribution of fluoride tolerance between algae and cyanobacteria, their physiological response differences, and possible mechanisms of action of fluoride in these groups. Algae and cyanobacteria differ in their response to fluoride, the latter being more sensitive to fluoride toxicity. The threshold concentration at which toxicity is manifested varies between organisms and is pH dependent. Being a strong hydrogen bonding species fluoride is capable of interacting with most of the cellular components and thus has a plethora of effects on cell metabolism.

Keywords: Algae, Cell membranes, Cyanobacteria, Fluoride toxicity, Metabolic processes, pH dependence

INTRODUCTION

Fluorine is the strongest oxidizing agent known. It occurs in natural waters as a free anion (F^-), undissociated hydrofluoric acid (HF), and its complexes.¹ The natural level of fluoride in uncontaminated fresh surface water is $<5 \mu M$, although industrial activities can increase concentrations to toxic levels. It is a major component of sea water where the concentration ranges from $1-3 \text{ mg kg}^{-1}$.² F levels equal to or exceeding $75 \mu M$ are hazardous to the marine environment,³ restricting the abundance and distribution of biota as has been observed by Pankhurst *et al.*⁴ Accumulation of fluoride and consequent impairment of normal functioning occurs both in plants^{5,6} and animals.^{7,8} Though voluminous information is available on the impact of fluoride on higher plants and animals, comparatively few studies have been reported on fluoride uptake and its effects on algae and cyanobacteria.

UPTAKE AND SENSITIVITY TO FLUORIDE

Among marine organisms, red algae are known to accumulate the highest levels of fluoride.⁹ Kesava Rao and Indusekhar¹⁰ reported $11.35-20.04 \text{ mg kg}^{-1}$ fluoride in red algae as compared to $4.78-17.82$ and $3.02-18.86 \text{ mg kg}^{-1}$ F in brown and green algae, respectively. This range of fluoride observed in algae was much higher than what occurs in land plants ($<10 \text{ mg kg}^{-1}$). Studies on the distribution of certain halogens in sea weeds show the equivalent concentrations of halogens in sea weeds was in the order $I > F > Br > Cl$, whereas in sea water the trend was $Cl > Br > F > I$.¹¹ No fluorine was detected in green algae even after 50 h of exposure to 2.5 mM fluoride but was accumulated in the cyanobacterium *Synechococcus leopoliensis*, *Oscillatoria limnetica*, and the diatom *Cyclotella meneghiniana* up to 54 ± 16 , 71 ± 17 , and $116 \pm 18 \text{ mg F.gDW}^{-1}$, respectively.¹²

Sensitivity to fluoride varies. Growth of *Chlorella pyrenoidosa* was reduced by 37% in the presence of $100 \mu M$ fluoride at pH 6.8,¹³ whereas 2.3 mM F proved to be lethal to *Scenedesmus* sp.¹⁴ Of the six freshwater planktonic mi-

^aFor correspondence: Department of Microbiology Kanad Vigyan Bhavan, Maharshi Dayanand Saraswati University, Ajmer (Rajasthan) India 305 009. Email: promicmon@yahoo.com, aridgabfa@hotmail.com

croalgae studied by Hekman, *et al.*,¹² only growth of the cyanobacterium *Synechococcus leopoliensis*, was transiently inhibited by addition of 2.6 mM NaF during the mid-logarithmic phase of the growth curve with resumption of growth at reduced F levels. They proposed that inhibition was probably due to a loss of cell potassium. Loss of up to 90% of cell potassium within a few hours of exposure to 7.9 mM F was shown by MacArthur *et al.*¹⁵ Resumption of growth was preceded by resorption of potassium from the medium. In general, cyanobacteria are highly sensitive to fluoride, whereas green algae appear almost resistant to F concentrations up to 10 mM.¹⁶ It has been suggested that the ambient fluoride concentrations observed in nature should have minimal effects on indigenous algal flora.

PH AND FLUORIDE

Fluoride toxicity appears to be a pH-related phenomenon. The synergistic effect of fluoride and low pH in reducing algal growth has been reported earlier by Nichol *et al.*¹⁷ for *Synechococcus leopoliensis* and *Oscillatoria limnetica* and on higher plants by Horner and Bell.¹⁸ The observed dependence of fluoride toxicity on pH may be a consequence of the pH-transformed chemical form of fluoride in solution. It has been hypothesized that F transport through biological membranes occurs primarily through non-ionic diffusion of HF¹⁹ that crosses five to seven orders of magnitude faster than the F ion (F⁻).²⁰ Since HF is a relatively weak acid (pK_a 3.45),²¹ significant concentrations of the HF molecule exist at physiological pH and increase as the pH falls. Thus it is the HF concentration and not the quantity of total fluoride which governs toxicity. HF concentration can be calculated from the Henderson-Hasselbalch equation: $\text{pH} = \text{pK}_a + \log(\text{F}^-/\text{HF})$. Based on this equation, threshold levels of approximately 1.1 μM HF for *S. leopoliensis*¹⁷ and 0.742 μM HF for *A. khannae*¹⁶ have been detected.

Tolerance of *Nitzschia palea* and *Amphora coffeaeformis* to high concentration of F at pH 7.6-8.2 has been reported by Joy and Balakrishnan.²² The authors suggested that the stimulation of growth up to 4.5 mM fluoride could be a result of F-induced increase in cell multiplication. These authors along with Oliviera *et al.*²³ suspected a nutritional role of F in algae. Similar growth enhancement using concentrations up to 2.5 mM F in *Plectonema* sp. and *Anabaena fertilissima*, have been reported by Bhatnagar¹⁶ who ascribed it to the phenomenon of hormoligosis.

Resistance to fluoride toxicity in microalgae has been correlated to be due to a lack of fluoride uptake.^{12,16,17} Nichol *et al.*¹⁷ isolated both resistant and tolerant forms of *Synechococcus leopoliensis* and observed that while the resistant cells show passive permeation of both F⁻ as well as HF across the cell membrane, the sensitive cells are permeable only to HF and thus accumulate fluoride to toxic concentrations.

Another mechanism of F resistance has been observed in *Chara fragilis* which can chemically combine fluoride into insoluble CaF₂ at the expense of CaCO₃.²⁴ Slow but successful adaptation to growth inhibitory concentrations of

fluoride accompanied by ultrastructural changes has been reported for *Amphidinium carterii* by Klut *et al.*²⁵

PHYSIOLOGICAL RESPONSES TO FLUORIDE

Algae and cyanobacteria show differential response to fluoride. Most of these effects are apparent at millimolar and lower concentrations. On the basis of available reports, a generalized speculative model of F action in the two groups is presented in the Figure.

MEMBRANE DAMAGE

Permeability and transport of fluoride across the cell membrane are the source of all the toxic effects of fluoride on the cell. Membranes can be affected either because insufficient energy is available for rapid synthesis of membrane components or because membrane lipids are hydrolyzed and used as alternative sources of energy.²⁶⁻²⁸ Since lipids are major components of the cell membrane, any change in the lipid or fatty acid composition can lead to altered membrane structure and function thereby causing greater electrolyte leakage.^{29,30} Heavy leaching of the electrolytes and phycobilins along with disruption of the cells of diazotroph *A. khannae* at higher F concentrations within a few hours of exposure to fluoride was indicative of a direct oxidative damage to the cell envelope. The effect accelerated with decrease in pH.¹⁶

A steady-state model for F transport across membranes and its subcellular partitioning was proposed by Miller *et al.*³¹ The basis of this model, as discussed earlier, resides in the different abilities of F⁻ and HF to pass through lipid membranes.²⁰ The charged ion has a permeability coefficient of 4.9×10^{-11} cm sec⁻¹, nearly 10⁶ times slower than HF that crosses a membrane at a rate of 1.4×10^{-4} cm sec⁻¹. Once in the cytoplasm, accumulating fluorides subsequently partition into the chloroplasts, mitochondria, and vacuoles, driven by transmembrane pH gradients and concentration differences. The transmembrane movement continues until organelle function is disrupted. Wei³² showed that fluoride at 10 mM concentration inhibits the maintenance of the transmembrane pH gradient. The cause of this disruption is blockage of chloride antiport movement inwardly during proton extrusion. Fluoride also interferes with the sodium pump distributing the equilibrium between intracellular potassium and extracellular sodium.³³

PHOTOSYNTHESIS

Fluoride is a classical inhibitor of algal photosynthesis. It enters cells of *Chlorella* at pH 4.0 and inhibits photosynthesis by limiting CO₂ utilization.³⁴ Vennesland³⁵ reported an immediate burst of CO₂ on addition of sodium fluoride to acid-grown *Chlorella*. Bishop and Gaffron³⁶ confirmed this and further showed that this CO₂ burst was probably a fluoride-induced decarboxylation of glutamic acid. The phenomenon however was not a generalized response to fluoride since several other algal forms such as *Chlorella*, *Scenedesmus obliquus*, *Ankistrodesmus braunii* and *Chlamydomonas moewusii* did not show fluoride-induced bursts of CO₂.

FIGURE. Speculative model proposing basis of differential fluoride activity in green algae (upper) and cyanobacteria (lower).

NOTE: pictures not displayed here.
Please see our membership page for subscription details to FLUORIDE.
<http://www.fluoride-journal.com/members.htm>



FIGURE LEGEND

1. Fluoride crosses the cell envelope in the non-ionic form HF (pK_a 3.45), which dissociates subsequently to form H^+ and F^- depending upon the intracellular pH. At low nontoxic dosages, it supposedly provides a chloride sparing effect and thus stimulates growth.
2. Single most important barrier to fluoride is the cell envelope (cell wall + cell membrane). Cellulosic cell wall of eukaryotic algae is much tougher than the gram-negative type cyanobacterial cell wall that offers more susceptible sites for direct F action.
3. Toxic action starts by damage to the transport channels associated with the cytoplasmic membrane. Being a very strong H-bonding agent, F^- is able to couple with virtually most of the cell constituents. It disrupts the cell envelope of the prokaryotic algae causing leakage of the cell material. It inhibits membrane-bound enzymes.
4. Driven by transmembrane pH gradients and concentration differences, partitioning of accumulated fluoride to subcellular organelles continues till organelle function is lost.
5. Other than thylakoid discs, prokaryotes have no internal membrane systems allowing direct exposure to fluoride. On the other hand the high level of internal compartmentalization in eukaryotes checks fluoride movement thus requiring a higher threshold level for F action.
6. Fluoride disrupts phycobilisomes from the photosynthetic apparatus in cyanobacteria leading to their leaching out of the cell.
7. Once inside the cytoplasm or an organelle, F^- has easy access to the enzyme systems, and some important respiratory and photosynthetic enzymes are inhibited. However, the pentose phosphate pathway, the major respiratory pathway in cyanobacteria, is stimulated while in eukaryotic algae matrix proteins of mitochondrion and presumably the enzymes of Krebs cycle are inhibited, thus having severe adverse effects.
8. Fluoride also modifies the ratio of free nucleotides and that of RNA, decreases the rate of RNA synthesis, and enhances RNase activity. As a result, it affects protein synthesis negatively.

Vennesland and Turkington³⁷ identified two loci of fluoride action – a primary locus in the light independent reaction of photosynthesis and a secondary site associated with the light-driven H₂O splitting Hill reaction, the latter being lesser sensitive. Sensitivity increased with decrease in pH. Increasing concentrations of CO₂ (optimum 0.5%) had a protective effect on the Hill reaction. Evidence for the action of fluoride on the light-independent reactions of photosynthesis was obtained for *Synechococcus leopoliensis* by Hekman *et al.*,¹² where photosynthetic O₂ release was inhibited by growth inhibitory F concentrations with marginal reduction in the Hill reaction.

Nichol *et al.*¹⁷ opined that photosynthesis was ATP limited in the presence of fluoride. Giannini *et al.*³⁸ reported interaction of fluoride with the thylakoid F₀F₁-ATPase of chloroplasts. Fluoride stress has been shown to cause shrinkage of thylakoids of the dinoflagellate *Amphidinium carterii*.²⁵ This effect is probably due to osmotic and/or molecular changes in its envelope membrane. Alternatively, the depletion of cellular ATP may be caused by a reduction in cytoplasmic pH which leads to activation of the plasma membrane proton extruding ATPase, thus causing ATP consumption. Such activation is well documented for weak acids.^{39,40} The consequent ATP depletion may affect a wide range of cellular functions like inhibition of phosphate uptake⁴¹ and also loss of K⁺ ions.¹⁵

RESPIRATION

Alterations in oxygen consumption and CO₂ evolution of plants exposed to fluoride result from changes in glucose metabolism caused by stimulation⁴² or inhibition^{43,44} of respiratory pathways. Respiratory inhibition starts at the mitochondrial membrane with restriction in the movement of Na⁺ and K⁺ ions⁴⁵ which, in turn, inhibits activity of systems necessary for the process. Membrane-bound enzymes such as ATPases are affected the most.

Regulation of respiration by the relative ratio of ADP/ATP has been suggested by Miller and Miller.⁴⁶ At 1.05x10⁻³ M fluoride, oxygen consumption in *Chlorella* increased to more than 107% and esterified phosphate increased by 160%.⁴⁷ This is indicative of multiple enzyme inhibition as proposed by Reiner.⁴⁸ However, the application of Reiner's theoretical treatment depends essentially on the increased esterified phosphates being ATP. Growth inhibitory NaF treatments have been shown to decrease the ATP level in the cells of *S. leopoliensis* by 75% and phosphate uptake by 95 - 97%.¹⁷

Enolase, the key enzyme of glycolytic pathways, has been shown to be F sensitive,^{12,49} although dark respiration was unaffected in the chlorophytes *Scenedesmus quadricauda* and *Ankistrodesmus braunii* studied by Hekman *et al.*¹² *Synechococcus leopoliensis* on the other hand showed a stimulation of O₂ uptake during a 5 h exposure to 2.6 mM fluoride, a growth inhibitory concentration. Its mechanism is not clear. Either wound respiration (a secondary response to F-induced physiological damage) or more directly, uncoupling of energy conservation from electron flow as proposed by Miller and Miller⁴⁶ for

F action on plants, may be involved. It is also possible that an inhibition of glycolysis is accompanied by the channeling of carbohydrates through the pentose phosphate pathway (PPP).⁵⁰ An increase in the relative proportion of PPP over glycolysis along with stimulation of G6P dehydrogenase has been reported in higher plants.^{51,52,53} The observed difference in the response of *Anabaena khannae* and *Chlorococcum humicola* was attributed to the difference in the respiratory metabolism of the two forms. In the green algae the process is similar to the higher plants being carried out via the Krebs cycle, while in cyanobacteria respiration occurs exclusively through the oxidative PPP.⁵⁴

Fluoroacetate-induced inhibition of endogenous respiration has also been reported.⁵⁵ In fluoride adapted *Amphidinium carterii*, strong evidence of changes in the mitochondrial matrix suggestive of competitive inhibition of matrix proteins and associated enzymes (presumably those of the Krebs cycle) were observed by Klut *et al.*²⁵ Many of the tricarboxylic acid dehydrogenases such as succinic, malic, and NADH dehydrogenases are sensitive to fluoride.^{56,57} Oxidative enzymes such as catalase, peroxidase, ascorbic acid oxidase, polyphenol oxidase, and cytochrome oxidase are stimulated.⁵⁸

NITROGEN FIXATION

Stress on the processes of photosynthesis and respiration leads to alterations in a number of other biochemical pathways. Diazotrophic cyanobacteria fulfill their N requirement by fixing atmospheric nitrogen through the molybdenum-requiring enzyme, nitrogenase. Inhibition of nitrogen fixation by fluoride has been shown in plants by Sheridan⁵⁹ and in *Nostoc calcicola* by Rai *et al.*⁶⁰ Bahal and Talpasayi⁶¹ reported heterocyst inhibition in *Anabaena ambigua* under F stress. A dose-dependent decrease in nitrogenase activity was observed in the cyanobacterium *Anabaena khannae*.¹⁶ The observed inhibition could be a result of direct involvement of fluoride with the enzyme or in part be due to alterations in photosynthesis and respiration that provide the necessary reductants and carbon skeletons for this energy intensive process.

CONCLUSION

Algae and cyanobacteria differ in their response to fluoride, the latter being more sensitive to fluoride toxicity. The single most important feature providing protection to the cell is the cell envelope. Once fluoride is inside the cell, the absence of internal compartmentalization in prokaryotic algae brings all the constituents under direct attack. Being a strong hydrogen bonding agent, fluoride has the ability to interact with most cellular components and thus shows a multipronged effect on cell metabolism. However, the threshold concentration at which toxicity is manifested varies between algae and cyanobacteria and is strongly pH dependent.

REFERENCES

- 1 Pitter P. Forms of occurrence of fluorine in drinking water. *Water Research* 1985;19:281-4.
- 2 Wilson TRS. Salinity and the major elements of sea water. In: Riley JP, Skirrow G, editors. *Chemical Oceanography Vol I*. New York: Academic Press; 1975. p. 365-413.
- 3 U.S.E.P.A. *Water Quality Criteria* 1972. Washington: Govt Printing Office; 1973. Report No. 73-033.
- 4 Pankhurst NW, Boyden CR, Wilson JB. The effect of fluoride effluent on marine organisms. *Environ Pollut. Ser. A* 1980;23:299-312.
- 5 Weinstein LH, Alscher-Herman R. Physiological responses of plants to fluorine. In: Unsworth MH, Ormrod DP, editors. *Effects of Gaseous Air Pollution in Agriculture and Horticulture*. London: Butterworths; 1982. p. 139-76.
- 6 McCune DC, Weinstein LH, Jacobson JS, Hitchcock AE. Some effects of atmospheric fluoride on plant metabolism. *J Air Pollut Control Assoc* 1964; 14:465-8.
- 7 Hemens J, Warwick RJ. The effect of fluoride on estuarine organisms. *Water Research* 1972;6:1301-8.
- 8 Bogin E, Abrams M, Avidar Y, Israeli B. Effect of fluoride on enzymes from serum, liver, kidney, skeletal and heart muscles of mice. *Fluoride* 1976;9:42-6.
- 9 Young GE, Langille WM. The occurrence of inorganic elements in marine algae of the Atlantic provinces of Canada. *Can J Bot* 1958;36:301-10.
- 10 Kesava Rao C, Indusekhar VK. Fluoride content of certain marine algae and sea water from Saurashtra coast (*Caulerpa scalpelliformis*, *Ulva lactuca*, *Codium dwarkensei*). *Indian J Mar Sci* 1984;13:47-8.
- 11 Kesava Rao C, Indusekhar VK. Distribution of certain cations and anions in sea weeds and seawater of Saurashtra coast and their geochemical significance. *Indian J Mar Sci* 1989;18:37-42.
- 12 Hekman WE, Budd K, Palmer GR, MacArthur JD. Response of certain fresh water planktonic algae to fluoride. *J Phycol* 1984;20:243-9.
- 13 Smith AO, Woodson BR. The effects of fluoride on growth of *Chlorella pyrenoidosa* Virginia *J Sci* 1965;16:1-8.
- 14 McKee JE, Wolf HW. *Water Quality Criteria*. 2nd ed. Resources Agency of California No. 3-A. Sacramento: State Water Resources Control Board; 1963. p. 140-1.
- 15 MacArthur JD, Palmer GR, Hekman WE, Budd K. Elemental analysis of algae with PIXE and PIGME. *Nucl Instrum Methods* 1983;218:519-24.
- 16 Bhatnagar M. Fluoride tolerance in microalgae and its ecological implications. (Dissertation). Indian Agric Res Inst: New Delhi; 1997.
- 17 Nichol BE, Budd K, Palmer GR, MacArthur JD. The mechanism of fluoride toxicity and fluoride resistance in *Synechococcus leopoliensis* (Cyanophyceae). *J Phycol* 1987;23:535-41.
- 18 Horner JM, Bell JNB. Effects of fluoride and acidity on early plant growth. *Agric Ecosyst Environ* 1995;52:205-11.
- 19 Armstrong WD, Singer L. Fluoride: Tissue distribution. Intracellular fluoride concentrations. *Proc Soc Exp Biol Med* 1980;164:500-6.
- 20 Gutnecht J, Walter A. Hydrofluoric and nitric acid transport through lipid bilayer membranes. *Biochim Biophys Acta* 1981;644:153-6.

- 21 Weast RC. Handbook of chemistry and physics. 59th ed. Cleveland: CRC Pub Co.; 1978. p. D203.
- 22 Joy OM, Balakrishnan KP. Effect of fluoride on axenic cultures of diatoms. *Water Air Soil Pollut* 1990;49:241-9.
- 23 Oliviera L, Antia NJ, Bisalputra T. Culture studies on the effects from fluoride pollution on the growth of marine phytoplankton. *J Fish Res Board Can* 1978;35:1500-4.
- 24 Levy LW, Strauss RL. Sur L' insolubilisation de L' Ion fluor a L' etat- de fluorure de calcium chez *Chara fragilis*. Desvaux CR. *Acad Sci* 1973;277:181-4.
- 25 Klut ME, Bisalputra T, Antia NJ. Abnormal ultrastructural features of a marine dinoflagellate adapted to grow successfully in the presence of inhibitory fluoride concentration. *J Protozool* 1981;28:406-14.
- 26 McCullough CH, Simon EW. The effect of iodoacetate on phospholipid levels and membrane permeability. *J Exptl Bot* 1973;24:841-6.
- 27 Zwiazek JJ, Shay JM. Sodium fluoride induced metabolic changes in jack pine seedlings. I. Effect on gas exchange water content and carbohydrates. *Can J Forest Res* 1988;18:1305-10.
- 28 Zwiazek JJ, Shay JM. Sodium fluoride induced metabolic changes in jack pine seedlings. II. Effect on growth, acid phosphatase, cytokinins and pools of soluble proteins, amino acids and organic acids. *Can J Forest Res* 1988;18:1311-7.
- 29 Zwiazek JJ, Shay JM. Fluoride and drought induced structural alterations of mesophyll and guard cells in cotyledons of jack pine (*Pinus banksiana*). *Can J Bot* 1987;65:2310-7.
- 30 Zwiazek JJ, Shay JM. The effects of fluoride on cytoplasmic leakage and the lipid and fatty acid composition of jack pine (*Pinus banksiana*) seedlings. *Can J Bot* 1988;66:535-45.
- 31 Miller GW, Pushnik JC, Giannini JL. A model for the subcellular distribution of fluoride in higher plants: Relation to *in vitro* concentrations required for enzyme inhibition In: Fluoride Research 1985. *Studies in Environmental Science* Vol 27. Amsterdam: Elsevier Science Publishers BV; 1986. p. 241-51.
- 32 Wei LL. Effect of hydrogen fluoride on ultrastructure of soybean leaf cells. (Dissertation). Utah State University; 1972. p.166.
- 33 Ballantyne DJ, Glover DL. Chloroplast electron transport protein and RNA in fluoride treated shoots. *Environ Exp Bot* 1981;21:83-8.
- 34 Warburg O. *New Methods of Cell Physiology*. New York: Wiley-Interscience; 1962.
- 35 Vennesland B. Photosynthetic mechanisms in green plants. Washington DC: Natl Acad Sci, Natl Res Council Pub. 1963;1145:421.
- 36 Bishop NI, Gaffron H. The inhibition of photosynthesis by sodium fluoride. 1. The sodium fluoride induced carbon dioxide burst from *Chlorella*. *Biochim Biophys Acta* 1958;28:35-44.
- 37 Vennesland B, Turkington E. The relationship of the Hill reaction to photosynthesis: Studies with fluoride poisoned blue-green algae. *Arch Biochim Biophys* 1966;116:153-61.
- 38 Giannini JL, Miller GW, Pushnik JC. Effects of NaF on the biochemical processes of isolated soybean chloroplasts. *Fluoride* 1985;18:72-9.

- 39 Sanders D, Hansen VP, Slayman CL. Role of the plasma membrane proton pump in pH regulation in non animal cells. *Proc Natl Acad Sci USA*. 1981; 78:5903-7.
- 40 Marre MT, Romani G, Coccuci M, Marre E. Internal pH and transmembrane potential as regulators of the activity of the proton pump of higher plants. *International Workshop on Membranes and Transport in Biosystems*, Bari, Italy: Laterza; 1982. p. 111-4.
- 41 Budd K, Kerson GW. Uptake of phosphate by two cyanophytes: Cation effects and energetics. *Can J Bot* 1987;65:1901-7.
- 42 Applegate GH, Adams DF. Effects of atmospheric fluoride on the respiration of bush beans. *Bot Gaz (Chicago)* 1960;121:223-7.
- 43 Hill AC, Pack MR, Transtrum LG, Winters WS. Effects of atmospheric fluorides and various types of injury on the respiration of leaf tissue. *Plant Physiol* 1959;34:11-6.
- 44 Pushnik JC, Miller GW. The influences of elevated environmental fluoride on the physiology and metabolism of higher plants. *Fluoride* 1990;23:5-19.
- 45 Miller GW, Yu MH, Pushnik JC. Basic metabolic and physiologic effects of fluoride on vegetation. In: Shupe JL, editor. *Fluorides: Effects on vegetation, Animals and Humans*. Proceedings of an International Symposium on Fluorides: 1982 May 24-7; Logan, Utah, USA. Salt Lake City: Paragon Press; 1983. p. 83-104.
- 46 Miller JE, Miller GW. Effect of fluoride on mitochondrial activity in higher plants. *Plant Physiol* 1974;32:115-21.
- 47 McNulty IB, Lords JL. Possible explanation of fluoride induced respiration in *Chlorella pyrenoidosa*. *Science* 1960;132:1553-4.
- 48 Reiner JM. *J Gen Physiol* 1947;30:367. (cited in reference no. 47).
- 49 Miller GW. Properties of enolase in extracts of pea seeds. *Plant Physiol* 1958; 33:199-206.
- 50 Ross CV, Weibe HH, Miller GW. Effect of fluoride on glucose catabolism in plant leaves. *Plant Physiol* 1962;37:305-9.
- 51 McCune DC. On establishment of air quality criteria with reference to the effects of atmospheric fluoride on vegetation. *Air Quality Monographs* 1969; 69:33.
- 52 Malhotra SS, Khan AA. Biochemical and physiological impact of major pollutants. In: Treshow M, editor. *Air pollution and plant life*. Chichester: Wiley; 1984. p. 486.
- 53 Miller GW. The effect of fluoride on higher plants with special emphasis on early physiological and biochemical disorders. *Fluoride* 1993;26:3-22.
- 54 Stanier RY, Ingraham JL, Wheelis ML, Painter PR. *General Microbiology*. 5th ed. London: MacMillan Education Ltd; 1987. p. 355.
- 55 Hochachka PW, Teal JM. Respiratory metabolism in a marine dinoflagellate. *Biol Bull* 1964;126:274-81.
- 56 Lovelace CJ, Miller GW. *In vitro* effects of fluoride on tricarboxylic acid cycle dehydrogenases and oxidative phosphorylation. Part I. *J Histochem Cytochem* 1967;15:195-201.
- 57 Lovelace CJ, Miller GW. Histochemical investigations on the *in vivo* effects of fluoride on tricarboxylic acid cycle dehydrogenases from *Pelargonium zonale*. Part II. *J Histochem Cytochem* 1967;15:202-6.

- 58 Lee CJ, Miller GW, Welkie GW. The effect of hydrogen fluoride on the wounding and respiratory enzymes in soybean leaves. *Int J Air Water Pollut* 1965;10:169-81.
- 59 Sheridan RP. Effect of airborne particulates on nitrogen fixation in legumes and algae. *Phytopathology* 1979;69:1011-8.
- 60 Rai R, Gupta SL, Kashyap AK. Inhibition of growth, nitrogen fixation and succinate TCA reductase in *Nostoc calcicola* by sodium fluoride: A pH dependent phenomenon. *Indian J Microbiol* 1980;20:201-3.
- 61 Bahal M, Talpasayi ERS. Cited in Reddy TRK, Sharda K. Effect of fluoride on growth and morphogenesis of *Anabaena* sp. In: Shukla AC, Pandey SN, editors *Advances in Applied Phycology. Proceedings of All India Applied Phycology Congress*; Kanpur, India. New Delhi: Today & Tomorrow's Publishers; 1983. p. 213-20.