SUMMARY AND CONCLUSIONS

Industrialization is essential for the development of nation, at the same time industries are responsible to add various kinds of pollutants in the environment. Thus environmental pollution is a burning problem all over the world. Fluoride is one of the pollutants. It is present in trace amounts in air, water and soil. It is not an essential element for plants, higher concentration is phytotoxic. The level of fluoride is increased in the environment by various anthropogenic activities. Plants absorb fluoride from soil, air, water and through food chain it may cause serious health problem to mankind namely fluorosis which affects the bone and teeth. In India, fluoride is detected in considerable amounts in various states, Gujarat being one of them. The effects of fluoride on plants is reported. The growth, physiological processes like photosynthesis, respiration, photosynthetic pigments and various enzymic activities as well as metabolites like sugar, amino acids, protein, phenol etc. are altered in plants treated with fluoride. Generally growth is inhibited by excess amount of fluoride. The inhibitory effects of fluoride are alleviated by salts of calcium and magnesium and also ascorbic acid. These compounds either decrease the absorption of fluoride or nullify the fluoride induced alterations in plants.

Radish is an important leafy vegetable where both leaf as well as root are used as salad and vegetable. It is raised through
seeds hence seed germination and seedling growth determine the productivity of the crop. Generally fluoride effects are studied in cereals, pulses but rarely in leafy vegetables like radish. Radish (Raphanus sativus L var Japanese White) was selected to study the fluoride induced alterations on growth and metabolism. Following were the aims:

i) To evaluate fluoride toxicosis in radish seedlings under laboratory conditions

ii) To find out biochemical markers of fluoride toxicity in radish seedlings and plants

iii) To assess fluoride toxicity in radish plants under field conditions

iv) To alleviate the fluoride toxicity by simple means with respect to growth and biochemical changes

To achieve the above mentioned aims, following experiments were planned.

EXPT I: STUDY ON FLUORIDE INDUCED ALTERATIONS ON GROWTH AND METABOLISM OF RADISH SEEDLINGS

Uniform graded seeds of radish var Japanese white were germinated in petri-plates lined with filter paper under laboratory conditions. The media for germination were DW, 200, 400, 600, 800 and 1000 ppm NaF. The percent germination was recorded from control and treated seedlings. 20 seedlings on completion of 48h, 72h, 96h and 120h from each treatment were studied for elongation of root, shoot, fresh weight, dry
weight and percent moisture. Fluoride effects were evaluated through R/S ratio, Relative Root Growth (RRG) and Relative Growth Rate (RGR). The seedlings were also analysed for carbohydrate metabolism (invertase activity, non-reducing sugar and reducing sugar), protein metabolism (protease activity, protein and amino acids), oxidizing enzymes (IAA oxidase, polyphenol oxidase and peroxidase) and phenolic compounds (total phenol content).

EXPT II: STUDY ON FLUORIDE INDUCED ALTERATIONS ON GROWTH AND METABOLISM OF RADISH

The uniform graded seeds of radish were sown on 5th Nov. 1992 in earthen pots. Before sowing the soil was mixed with varying amounts of NaF i.e. 200, 400, 600, 800 and 1000 ppm. The pots without NaF were considered as control. Plants were raised using normal practice. NaF induced alternations on growth and metabolism were studied. The experiment was repeated in the following year with change in sowing date i.e. seeds were sown on 15th Oct. 1993.

10 plants at random from each treatment i.e. control, 200, 400 and 600 ppm NaF (growth was not possible beyond this concentration) were studied for growth parameters (root length, shoot height, fresh wt. and dry wt. of root, shoot and whole plant as well as leaf no.). The metabolism study includes carbohydrate metabolism (invertase activity, non-reducing sugar, reducing sugar), protein metabolism (protease activity, protein, amino acids and proline), oxidizing enzymes
(IAA oxidase activity) and phenolic compounds (total phenol content) from root and leaf. The study was carried out at fortnightly intervals upto 60 days.

The growth parameters were studied from both the years while metabolism study was carried out from the first year of sowing.

**EXPT III: STUDY ON EFFECTS OF NaF+MgCl₂ ON GROWTH AND METABOLISM OF RADISH SEEDLINGS**

Uniform graded seeds of radish were germinated in sterilized petri plates lined with filter paper under laboratory conditions. The media used for germination were DW, 400 ppm NaF, 400 ppm MgCl₂ and 400 NaF+MgCl₂ each. The growth and biochemical parameters were studied as mentioned in expt I.

**EXPT IV: STUDY ON EFFECTS OF NaF+MgCl₂ ON GROWTH AND METABOLISM OF RADISH**

The uniform graded seeds of radish were sown in earthen pots on 5th Nov. 1992. Before sowing the soil was mixed NaF (400 ppm), MgCl₂ (400 ppm) and NaF+MgCl₂ (400 ppm each). The pots without NaF or MgCl₂ were considered as control. The plants were raised using normal practice. The experiment was repeated in the following year with change in sowing date i.e. seeds were sown on 15th Oct. 1993. The growth parameters were studied from both the years while metabolism study was carried
out from the first year. The parameters were same as mentioned in expt II.

The following conclusions were drawn from the above experiments.

1. When seed of radish var Japanese white were germinated in DW, and NaF (200, 400, 600, 800 and 1000 ppm), percent germination was delayed and reduced by sodium fluoride. The inhibition was correlated with concentration.

2. Elongation of root and shoot was reduced by fluoride. The adverse effect was positively correlated with concentration. The root elongation suffered more than shoot elongation especially in older seedlings. R/S ratio was also lowered by NaF. Higher concentration was more effective.

3. Fresh weight of radish seedlings was lowered by NaF. Adverse effect progressively increased with increase in concentration. Dry weight was higher in 120h old NaF grown seedlings in comparison to that in DW grown seedlings. Water uptake was lowered by NaF, lowering was positively correlated with concentration.

4. NaF stimulated the invertase activity, stimulation was concentration dependent. Significant effect was noted
during first 24h of germination. Fluoride lowered the non-reducing sugar, effect was significant in older seedlings. Reducing sugar was more in NaF treated younger seedlings, longer duration of treatment lowered the sugar level. The effect of NaF on sugar level varied directly with concentration.

5. The protein metabolism in terms of protease activity, protein content and total amino acid content was lowered by NaF. The depression in protein metabolism was correlated with concentration. Changes were sharp in amino acid content. The determination of amino acids may be considered as biochemical marker for assessing fluoride toxicity at seedling stage.

6. The oxidising enzymes namely polyphenol oxidase and IAA oxidase were lowered by NaF while peroxidase activity was stimulated in radish seedlings. Total phenol was lowered by NaF. The effects of fluoride on oxidizing enzyme and phenol were positively related with concentration and duration of treatment.

7. When NaF was used as soil pollutant (200, 400 and 600). The elongation of root and shoot of radish plants grown in Nov. 1992 was decreased by fluoride. The decrease was positively related with concentration. Adverse effect was more on root than shoot. The effect of fluoride was not visible on leaf number, though in 30
days old plants leaf number was decreased by higher concentration of NaF.

8. Fluoride lowered the fresh weight of root & shoot. Lowering was visible after 30 days in case of root while after 45 days in case of shoot. The increasing dose accelerated the adverse effect. Root and shoot of old plants were equally affected by fluoride.

9. Fluoride decreased the dry matter of root, shoot and whole plant; shoot was highly affected. Decrease was correlated with concentration. Percent allocation was altered by fluoride, it was enhanced in root and lowered in shoot. Effect was concentration dependent.

10. Percent moisture in root and leaf in younger and older plants was affected by fluoride. Higher concentration was more effective. Generally in the root it was lowered but in the shoot it was enhanced by NaF.

11. When effects of fluoride on elongation of radish which was raised through early sowing i.e. 15th Oct. 93, was studied, graded doses of fluoride lowered the elongation of both the organs. The effect was concentration dependent. Root was affected more than shoot. Leaf no. was less in 60 days fluoride treated plants.
12. The fresh wt. of root, shoot and whole plant was lowered by 400 and 600 ppm NaF. Shoot was affected more than root. 200 ppm enhanced the root fresh weight.

13. Fluoride lowered the dry weight of root, shoot and whole plant though 200 ppm NaF enhanced the dry matter of root in 60 days old plants. 400 ppm and 600 ppm NaF concentration gave similar adverse effect on shoot of 60 days old plants. The adverse effect on root and whole plant was highly correlated with concentration.

14. Fluoride stimulated the water uptake in root, shoot and whole plant. 600 ppm gave significant effect in 15 days old plants.

15. The invertase activity, non-reducing sugar and reducing sugar were higher in root and leaf of fluoride treated plants than that in control. The increase was positively correlated with concentration.

16. Fluoride stimulated the protease activity in root and leaf. Stimulation was positively correlated with concentration. Protein level was lowered by fluoride. Lowering was significant in root. In 60 days old plants higher protein was found in fluoride treated plants.

17. Fluoride decreased the amino acids. Decrease was significant in the leaf. Higher concentration was
highly effective. Proline was accumulated in root and leaf of fluoride treated plants. Accumulation was positively correlated with concentration of applied fluoride.

18. IAA oxidase activity in root and leaf was decreased by NaF. Inhibitory effect was related with concentration. Root was affected more than leaf. Higher phenol was found in treated plants in comparison to that in control plants. Moreover accumulation was significant in root than in leaf and it was correlated with concentration.

19. Chlorophyll 'a', Chlorophyll 'b', total Chlorophyll and carotenoids were more in fluoride treated plants. The effect was directly proportional to concentration and was significant in older plants.

20. When radish seeds were germinated in DW, 400 ppm NaF, 400 ppm MgCl₂, and 400 ppm NaF+MgCl₂ (each) under laboratory conditions the inhibitory effect of NaF on seed germination was alleviated by MgCl₂. The percent germination was stimulated by MgCl₂.

21. Elongation of root and shoot was lowered by 400 ppm NaF, lowering was less in seedlings treated with NaF+MgCl₂.

22. Fresh weight of seedlings was lowered by NaF. Lowering was less when seeds were germinated in NaF+MgCl₂. Dry
weight of seedlings remained higher in NaF and NaF+MgCl₂ treatments. Percent moisture was less in these seedlings.

23. NaF stimulated the invertase activity in radish seedlings, this was reversed by addition of MgCl₂ to NaF. The non-reducing sugar and reducing sugar were decreased by NaF in older seedlings. Effect were partially overcome by MgCl₂.

24. Protease activity and protein were lowered by NaF MgCl₂ as well as NaF+MgCl₂ stimulated it. Amino acids were lowered by NaF, the effect was totally reversible by addition of MgCl₂.

25. Polyphenol oxidase was higher in NaF treated seedlings than that in control. The activity was more or less similar in control and MgCl₂ treated seedlings but MgCl₂ alongwith NaF enhanced it. Fluoride stimulated the peroxidase activity MgCl₂ did not cause significant effect but NaF+MgCl₂ stimulated it, however compared to NaF treatment the stimulation was less. IAA oxidase activity was suppressed by fluoride. Suppression was less when MgCl₂ was added to NaF during germination. MgCl₂ stimulated the IAA oxidase activity in younger seedlings. Phenol content was lowered by NaF, MgCl₂ and NaF+MgCl₂ treatment. Lowering was significant with fluoride.
26. When radish plants were raised with NaF, MgCl₂ and NaF+MgCl₂ (400 ppm each) in Nov. 1992, NaF as a soil pollutant lowered root & shoot elongation, severe effect was found on root. Presence of MgCl₂ and NaF+MgCl₂ in soil also inhibited the root shoot elongation but on completion of 60 days elongation was improved and effects of fluoride were nullified. Leaf number was decreased by NaF, MgCl₂ and NaF+MgCl₂ in 30 days old plants. Later on leaf number were same in control and treated plants.

27. Fluoride lowered the fresh weight of root, shoot and whole plant. Lowering was visible after 30 days. Effect on shoot fresh weight was much significant compared to root. Mg enhanced the fresh weight in 60 days old plants. When MgCl₂ was added to NaF, inhibitory effect was slowly removed. In 60 days old plants, fresh weight was slightly more in NaF+MgCl₂ treated plants than that in control.

28. The inhibitory effect of fluoride on dry weight of root, shoot and whole plant was not visible in 60 days old plants treated with NaF+MgCl₂. Presence of Mg in the soil significantly increased the dry wt of both the organs.
29. Percent moisture in root of 15 days old plants was lowered but in shoot it was enhanced. The effect was reversible by addition of MgCl₂.

30. In the year 1993-94 when plants were raised through October sowing, Mg improved the root shoot elongation. Leaf number was not affected. The inhibitory effect of fluoride on root shoot elongation was alleviated by Mg.

31. Fluoride lowered the fresh weight of root, shoot and whole plant. It was more or less similar in control, MgCl₂ and NaF+MgCl₂ treated plants.

32. Fluoride decreased the dry weight, shoot was severely affected. Mg counteracted the effect. The dry wt. of MgCl₂ and NaF+MgCl₂ treated plants were more or less similar.

33. Fluoride generally enhanced the moisture level in root, shoot and whole plant. It was more or less similar in control, MgCl₂ and NaF+MgCl₂ treated plants.

34. Invertase activity in root and leaf was stimulated by 400 ppm NaF as well as NaF+MgCl₂. MgCl₂ was most effective followed by NaF+MgCl₂ and NaF. Fluoride increased the level of non-reducing and reducing sugar in both the organs, Mg partially counteracted the effects. Counteracting effect was significant in the leaf.
35. NaF, MgCl₂ and NaF+MgCl₂ stimulated the protease activity in root and leaf. Stimulation was significant in root. MgCl₂ was most effective and its effect were found even in plants treated with NaF i.e. Mg counteracts the effect of fluoride. Fluoride decreased the protein in root and leaf while MgCl₂ enhanced it. The adverse effect of fluoride on protein was totally counteracted when fluoride was supplemented with Mg. NaF, MgCl₂ and NaF+MgCl₂ decreased the amino acids in both the organs. The inhibitory effects were partially alleviated in root but totally alleviated in the leaf after 30 days. Proline was accumulated in root and leaf of fluoride treated radish. The effects were partially overcome by Mg.

36. IAA oxidase activity in root and leaf was lowered by fluoride. Such lowering was not observed in the plants treated with NaF+MgCl₂. Level of phenol was higher in plants treated with NaF and NaF+MgCl₂ than that in control, it was much more in NaF+MgCl₂.

37. Chlorophyll 'a' and total chlorophyll were higher in the plants treated with MgCl₂ as well as NaF+MgCl₂. Fluoride enhanced the chlorophyll 'b' and carotenoids significantly. The effects were partially reversed by MgCl₂.
38. Fluoride was taken up by root and leaf of radish grown on fluoride contaminated soil. It was more in root than in leaf. The uptake was concentration dependent. The uptake was reduced when fluoride was supplemented with MgCl$_2$.

Finally it is concluded that NaF inhibited the growth of radish, however there were no symptoms of chlorosis or necrosis. Inhibitory effect was concentration dependent. Addition of MgCl$_2$ to NaF, alleviated the toxic effect of fluoride on growth of radish. Fluoride altered the various metabolic processes in seedling, root and leaf of radish. Biochemical changes in sprouting seeds predict the fluoride toxicity. The significant decrease in level of amino acids in fluoride treated plants can be considered as biochemical marker for fluoride toxicity while higher level of sugars, proline and photosynthetic pigments suggests survival strategy against fluoride pollution. Counteracting effects of Mg on various metabolic processes were noted in seeds/plants treated with NaF+MgCl$_2$. Fluoride was found in root and leaf of radish grown in fluoride contaminated soil.

Thus it can be recommended that salts of magnesium should be added to fluoride contaminated soil in order to the detoxify the fluoride. The presence of fluoride in plants without visible symptoms indicates that regular intake of such plants should be avoided. Fluoride should be estimated from edible plants before human consumption.