FLUORIDE CONTENT OF FOOD AND POTABLE WATER AND ITS INTAKE IN ADULTS OF A FLUOROTIC VILLAGE

By

POONAM SHARMA

Thesis submitted to the CCS Haryana Agricultural University in partial fulfilment of the requirements for the degree of :

MASTER OF SCIENCE

IN

FOODS AND NUTRITION

I.C.COLLEGE OF HOME SCIENCE
CCS HARYANA AGRICULTURAL UNIVERSITY
HISAR
1993

DEDICATED

TO

MY BELOVED PARENTS

CERTIFICATE-I

This is to certify that this thesis entitled " Fluoride content of food and potable water and its intake in adults of a fluorotic village " submitted for the degree of M.Sc. in the subject of Foods and Nutrition of the CCS Haryana Agricultural University is a bonafide research work carried out by Miss Poonam Sharma under my supervision and no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

18/5/93

(Dr. Mrs. Usha Mehta)
Major Advisor,
Department of Foods and Nutrition,
CCS Haryana Agricultural University,
Hisar-125 004

CERTIFICATE-II

This is to certify that the thesis entitled " Fluoride content of of food and potable water and its intake in adults of a fluorotic village" submitted by Miss Poonam Sharma to the CCS Haryana Agricultural University in partial fulfilment of the requirements for the degree of Master of Science in the subject of Foods and Nutrition has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.

11/8/93
MAJOR ADVISOR

11.8.93
EXTERNAL EXAMINER

HEAD OF THE DEPARTMENT

DEAN, POST-GRADUATE STUDIES

ACKNOWLEDGEMENTS

It is my great previlege to express my heartfelt gratitude and sincere thankfulness to my major advisor Dr. (Mrs.) Usha Mehta, Associate Professor, Department of Foods and Nutrition, for her unceasing guidance, inexaustible encouragement, timely legitimization and an enduring as well as sympathetic understanding during this investigation.

I am highly grateful to the members of my advisory committee namely Dr. P.D.Puri, Professor and Head, Department of Statistics, Dr.(Mrs.) Salil Sehgal, Associate Professor of Foods & Nutrition, Dr. (Mrs.) Sarla Malhotra, Associate Professor of Biochemistry, Dr. O.P.Yadav, Associate Professor of Chemistry for their constructive criticism and giving precious suggestions despite of the pressure of their regular work.

I acknowledge my indebtedness to Dr. B.M.Chauhan, Dean, College of Home Science and Professor of Foods and Nutrition, Dr. A.C.Kapoor, Professor and Head, Department of Foods and Nutrition, Dr. Anoop Singh, Professor, Department of Soils, whose magnanimous help and keen interest in my research work served as a source of perpetual inspiration to attain the goal.

My sincere appreciation goes to my friends Manjeet Kaur, Kiran, Bharti, Sangeeta, Shiwani, Shalini, Alka Sharma and all the faculty members of Department of Foods and Nutrition for providing me with every necessary help and a nice companionship.

I feel paucity of words to express my eternal feelings for my great parents, Uncle, Dr. D.T.Sharma, Dr. Pasricha, Dr. V.P.Sharma and my friend Dr. Nasir Ali for their deep affection patronage, patience and tireless efforts in enamouring me to succeed in this endeavour. I will be failing in my duties, if I do not extend my special gratitude to my brothers and sisters for their innocent prayers.

I feel obliged to the United Nations Development Programme, for the award of Senior Research Fellowship to persue this study.

Poonam
(POONAM SHARMA)

HISAR

Dated : May 18 , 1993

CONTENTS

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LIST OF TABLES

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List of Illustrations

CHAPTER-I

INTRODUCTION

Endemic fluorosis with neurological, skeletal and gastrointestinal involvement has been recognised as a public health problem in India (Susheela, 1990; Somvanshi et al., 1990). Fluorosis is generally caused by ingesting excessive amounts of fluoride. The major clinical manifestations are severe pain in the backbone (vertebral column), joints and pelvic leading to stiffness of vertebral column and immobile joints terminating in a crippling condition (Susheela, 1984). Other features of this disorder may be muscular weakness, loss of muscle power, excessive thirst anorexia, diarrhoea, chronic constipation (Krishnamachari et al., 1974; Jolly et al., 1969; Teotia et al., 1979; Chandra, 1985).

Incidence of food and water borne fluorosis has been reported in many parts of the world like USA, Italy, Holand, Germany, China, several other African and South American countries and India. In India the endemicity of fluorosis has already been established in 12 out of 22 States. Haryana is one of them. Ten districts (Fig. 1) of Haryana State have been further identified to be endemic for fluorosis (8th Five Year Plan Document), Hisar being one of them. The districts of Rewari, Faridabad, Sonepat, Gurgaon, Mohindergarh have been reported to have 1.2 to / 6 ppm ground water F' levels. Reports of water F' levels of other districts are still awaited.

The water technology mission has provided schemes for alternate water provision for 18 villages of District Gurgaon. The State Level Committee regarding health awareness and problems associated with high F' in drinking water has emphasized the need of information on food habits and dietary factors of the rural population in relationship to the prevalence of incidence of fluorosis (Minutes, State Level Committee Meeting on Fluorosis, 19.1.1993).

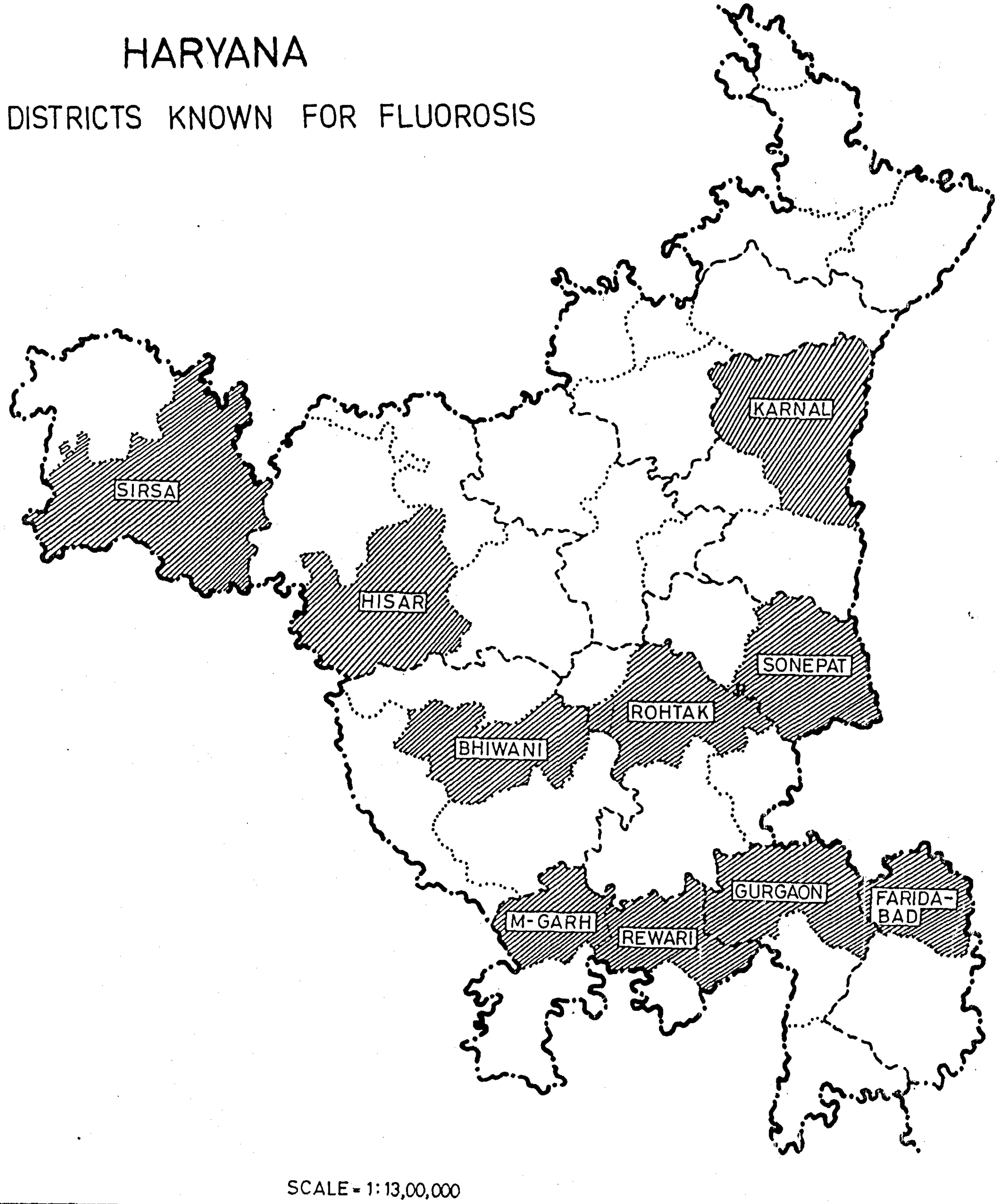

Fig. 1. MAP OF HARYANA SHOWING THE 10 DISTRICTS WHICH ARE KNOWN TO BE ENDEMIC FOR HYDROFLUOROSIS.

The primary cause of endemic fluorosis has been established to be prolonged consumption of excessive intake of trace element fluoride (F') primarily through drinking water. The ground water fluoride levels are high in areas which are largely rocky and sandy with hot dry climates and low rain fall (Raju, 1983). The depth of the drinking water source is also known to influence the water F' level. The water from deep bore sources are seen to contain lower F' levels than shallow ones (Teotia, 1987; Shukla, 1982; Reggabi and Poey, 1983). The occupation of the people and their vulnerability of fluorosis are also related. Since, the disease was found to be more common in farmers and heavy manual workers especially those carrying heavy loads on head. This may probably be due to increase strain of body parts leading to increased blood supply resulting in greater local accumulation of fluoride (Shukla, 1982). Studies are also available to indicate the sexual differences in the degree of proneness to fluorosis wherein the males have been shown as more vulnerable to this abnormality than the females (Jolly et al., 1969; Shukla, 1982). Poor nutritional status and inadequate dietary protein, calcium and vitamin 'C' also make people more susceptible to fluorosis (Krishnamachar et al., 1978).

Currently, certain consumer goods like mouth washes (rinses) toothpastes etc. are being extensively and highly fluoridated. Betal and tobacco chewing are also important sources of fluorine intake reported by Nanda, 1972. Similarly, tea, spices and green leafy vegetables also contain very high levels of fluorides (Szpuner et al., 1988; Horowitz, 1989).

The Haryana State is by and large rocky and sandy ones with hot climate and low rainfall. Soils are calcareous and usually a massive Kankar pan at depth of 0.75 to 1.25 m. Salinity and alkalinity are the serious problems, responsible for high F' level. The rural population of Haryana villages mainly use ground water from wells and hand pumps for drinking and cooking despite the piped water being available in each village. Thus amongst the factors predisposing villagers to fluorosis are potable ground water, substantial prevalence of malnutrition, high tea consumption, tobacco chewing and smoking. Like any other states of India, Haryana is also a predominantly agriculture dependent state involving lot of hard labour of the people.

There are some reports wherein the urinary F' excretion is strongly and positively correlated with F' intake of individuals (Zipkin and Leone, 1957; Mingxuan et al., 1984; Gupta et al., 1988; Chinoy et al., 1992). While studies on the comparison of urinary F' excretion of fluorotics with normal subjects reveal no difference (Mingxuan et al., 1984). Other studies (Teotia et al., 1952 ; Reddy et al., 1983; Rajalakshmi, 1984) revealed that the severity of the disease and urinary F' excretion have no correlation.

With this background, it was decided to study raw foods F' contents and compare the total F' intakes and urinary F' excretion of the fluorotic and normal rural adults of village Gangwa near Hisar city. The observations on the seasonal variation in all these parameters of both the fluorotic and normal adults is also required for more insights into the etiology of fluorosis. This could also be facilitated by knowledge of the difference of fluoride ingested through different food components by the fluorotic and the normal adults during the different season of the year. The objectives of the study therefore were :

1- To determine the raw foods and potable water F' levels; and

2- to compare the F' intake and urinary excretion of the fluorotic and normal adults.

CHAPTER-II

REVIEW OF LITERATURE

The literature regarding different aspects of fluorosis in humans has been reviewed under the following aspects :

2.1 Fluoride requirements

2.2 Fluoride content of :

2.2.1 Potable water

2.2.2 Food

2.2.3 Salt

2.2.4 Tea

2.2.5 Toothpastes

2.3 Urinary fluoride excretion

2.1 Fluoride requirements :

Fluorine is one of the most abundant element in the earth's crust and is distributed ubiquitously throughout nature. The presence of F' in biological materials is of considerable significance nutritionally because of its beneficial effects at moderate levels of intake and toxic effects at higher levels of intakes.

Longwell (1957) reported the total amount of F' intake to be 3.2 and 2.2 mg per day for men and women, respectively in some areas of United Kingdom where the water fluoride level was 1 ppm.

Ericson (1970) observed that fluorine is required in very small amounts by the body and excess dose results in fluorosis marked by changes in teeth and skeletal system.

Horowitz (1973) recommended the optimum F' level for reduction of dental caries without undesirable mottling is 1 ppm F' for temperate climates, providing a total daily intake of 0.5 to 1 mg F' per day for children during the period of tooth formation and 1.5 to 2 mg F' per day for adults. Water fluoridation provides an optimal F' intake for more than 150 million people throughout the world, including almost 50 per cent of the population of U.S.

Kramer et al. (1974) stated that intake of F' by adults from foodstuffs (exclusive of drinking water) averages approximately 1 mg per day in non-fluoridated communities and 2 to 3 mg per day in fluoridated communities (1 ppm F') in the United States.

Farkas and Farkas (1974) determined the optimum safe daily dose of F' as 0.05 - 0.7 mg/Kg body weight in children. According to WHO report (1986) the total fluoride intake varies from 0.2 mg of F' per day in infants to 5.0 mg of F' per day in adults.

National Research Council U.S.A. (1980) recommended daily F' intake of 1.5 mg to 4 mg for adults; 2.5 mg for children and adolescents from 4 years onward; 0.1 to 1 mg during first year of life followed by 0.5 mg to 1.5 mg during subsequent 2 years.

Teotia and Teotia (1984) reported the maximum permissible safe limit of F' in drinking water to be 1.0 ppm but should be reduced to 0.5 ppm due to epidemiological studies results. At 0.6 ppm, both dental and skeletal fluorosis have been observed in the residents of rural areas. The safety limit of F' in drinking water recommended by western countries does not apply to us because of our smaller skeleton size, intake of much higher volumes of water due to hot climatic conditions and probably the widespread malnutrition, therefore, for India the optimum range of F' in drinking water is around 0.5 ppm to prevent tooth decay and dental caries. No recommendations with regards to the dietary allowances have so far been made by the ICMR for Indians.

2.2 Fluoride content of :

2.2.1 Potable water :

Fluorides are widely distributed in nature and universally present in various amounts in soil, water, atmosphere, vegetations, sea foods and animal tissues.

The amount of F' ingested through the drinking water determines the risk of developing fluorosis, the concentration of F' and duration of high F' water intake being the critical determinants of fluorosis.

Singh et al. (1962) analysed F' content of 60 water samples from village Bajekhana of district Bhatinda (Punjab) and reported it to vary from 2.4 to 16.2 ppm with a mean of 8.4 ppm. Anand et al. (1963) in a study of Bindupur village near Delhi reported the well water F' content to be between 0.4 to 4.0 ppm.

Ramamohan and Bhaskaran (1964) analysed 302 water samples of 44 localities of Karnool district of Andhra Pradesh and reported to contain 0.1 to 6.0 ppm F'.

Dhar (1971) observed that in village Rahimabad, Lucknow, the water F' content from shallow wells ranged from 0.1 to 1.2 ppm and in hand pumps it was below 1.0 ppm.

Nanda and Kapoor (1972) analysed 100 well water samples for their contents of fluorine, phosphate, magnesium, calcium, chloride and hardness and tried to find if there were seasonal differences in the concentration of these constituents in sedimented and their respective clear samples. They further determined that F' in monsoon; fluorine, phosphate and magnesium in winter sediment samples were significantly higher.

Tamboli et al. (1980) reported that F' content in well waters ranged from 14.3 and 13.9 mg/litre in Pratabpura and Surajpura villages in Ajmer district, Rajasthan.

Ray et al. (1983) studied 41 wells in Rustampura and Ledhupur villages of Varanasi (U.P.) for F' content. The water samples were analysed during summer and post rainy seasons in 27 wells only. There were no significant differences in F' level of water collected in two seasons in either of two villages (t = 0.33; d.f. 26 : p $\angle$ 0.05). Nair and Gitonga (1983) found that in Kenya the underground water F' levels varied from 0.1 to 100 ppm and the surface water contained a maximum of 34 ppm. Over 30 per cent of Kenya population was found to be suffering from fluorosis.

In an Uttar Pradesh village, near Varanasi the well water F' content ranged from 0.2 to 2.1 ppm. The association between dental fluorosis and drinking water was positive (Ray, 1983).

The F' concentration in ground water was reported to be as high as 12.0 ppm in parts of Andhra Pradesh. The level was reported to be high in canal water due to Kankar rich in $Caco_3$ (Raju, 1983).

A study carried out in Northern parts of Sahara in Algeria indicated ground water F' content variation to be 0.2 to 5.4 ppm and 0.25 to 2.75 ppm F' from deeper water (Reggabi and Poey, 1983).

Susheela (1984) reported F' content of water samples to be ranging from 0.4 to 8 ppm from the various sources viz., open wells, tubewells, ponds and wells in Libya and Lathi taluks villages in Amerli district of Gujarat.

In a study carried out near fluorospar mines in Gujarat the drinking water F' content was found to vary from 0.4 to 3.25 ppm in 24 tribal villages (Desai et al., 1986).

Teotia et al. (1987) carried out a study in five villages in Meerut district and two villages in district Rai Bareli and reported that deep bore drinking water is a practical approach for eradication of endemic fluorosis in India.

Gupta (1988) reported that the ground water F' contents from all over Hisar town varied from 0.05 to 14.6 ppm.

Horowitz (1989) reported the concentration of F' in drinking water to be the major determinant of the prevalence and severity of the disease.

Fellensfeld et al. (1991) reported F' content of well water to be 428 u mole/litre in some parts of United States against the recommended levels of 11 to 58 u mole/litre. F' levels were greater than 212 u mole/lire in 3 of 12 wells of similar depth. They concluded that in areas known to be endemic for fluorosis, fluoride concentration of well water should be measured at the time of drilling.

Gikunju et al. (1992) reported that high F' levels in drinking water were associated with dental fluorosis. The maximum concentration of F' was 32 ppm while the lowest value was 0.13 ppm. They concluded that borehole water may be potential health hazard to consumers and therefore F' levels should be analysed before being recommended for domestic or individual use.

2.2.2 Fluoride content of food :

Fluorine, a highly active element is absorbed by the system through various sources including the diet.

Underwood (1977) estimated the F' intake and found it to be as low as 0.5 mg/day in adults. Intakes may vary widely depending on the water supply which may contain 0.1 to 40.0 ppm F'.

Lakshmaiah and Srikantia (1977) reported that sorghum based diets resulted in higher retention of F' as compared to rice or wheat based ones.

Duckworth and Duckworth (1978) reported that the daily ingestion of F' by tea drinkers of all ages varied from 0.04 to 2.7 mg in United Kingdom.

Sherlock (1984) found that tea contributes 1.3 mg to the average daily F' intake of 1.8 mg in U.K.. In Great Britain consumption of tea resulted in F' intakes as high as 8.9 mg/day. Walters et al. (1984) reported that beverages, particularly, tea account for 71 per cent of F' intake in U.K.

Taves (1983) analysed composite diets of adults and reported the daily intake level to be between 1.21 and 2.0 mg/day. Daily F' intake from food was 0.4 mg, equivalent to about 25 per cent of total intake of 1.8 mg. Walter's et al. (1984) also estimated that F' in diet and reported that the average dietary intake per person in the United Kingdom was 1.82 mg. Mean F' intake was 0.418 mg/d (0.052 mg/kg body weight) and 0.621 mg (0.050 mg/ kg body weight) for 6 months old child and 2 year old child drinking water with / 0.7 ppm F', respectively.

Tsunoda and Tsunoda (1985) found that the average daily intake of F' by each subject was 7.84 mg followed by ingestion of rice and green leafy vegetables produced in an area known to be polluted by air borne fluoride, while it was 1.89 mg when they were on controlled menu.

Sangha et al. (1991) carried out a study in rural areas of Faridkot district of Punjab which revealed the average daily F' intake from all the sources including food, water and tea to be 19 mg in men and 13 mg in women. The F' concentration of drinking water was found to be more than the recommended level (2.69±1.50 ppm).

Rao et al. (1991) reported total daily dietary F' intake from food and drinking water in rural south population to be 0.2 to 7.3 mg (0.05 to 0.32 mg/kg body weight).

Fluoride content of other miscellaneous items :

Lakdawala (1973) reported that milled procedures to increase F' concentration in wheat and Bengal gram flours. This is probably due to cont contamination from local grinders made of corrandom stone which contains F' compounds.

Nanda and Gitonga (1983) in a study of North Indian (Lucknow) cooked food reported that high F' content in cooked foods was due to the water, salt and spices used in cooking. The F' content of bengal gram was maximum (3 ppm) and the uncooked vegetables, fruits and fats were not rich sources of F'.

Gupta (1988) analysed F' contents of three green leafy vegetables and reported F' contents of spinach, cabbage and chulai to be 4.6, 1.64 and 17.02 ppm, respectively.

Schamschula et al. (1988) analysed more than 750 raw and cooked food samples in 3 areas of Hungary where F' concentration in water were 0.06 to 0.11, 0.5 to 1.1 and 1.6 to 3.1 mg/litre. Fruits and vegetables grown in 3 areas did not reflect the differences according to the F' concentration in water. The effect of high F' water used in food preparations was clearly discernable as increased amounts of F' in soup, parboiled rice, potatoes and noodles and also possibly in bakery products. The concentrations were increased in some meat products especially when bone dusts or fragments were included in the product.

Wakode (1990) analysed the F' content in foodstuffs grown in an endemic fluorotic area in Vidharbha (Dongargaon) in Maharashtra and reported notably high F' content of red gram (11.20 ppm), red chillies (10.9 ppm), beans (6.90 ppm), jowar (6.2 ppm) and Brinjal (3.90 ppm).

Rao et al. (1991) analysed F' content of some common south Indian foods and their contribution to fluorosis. Out of 18 food items, with special reference to feeding habits of remote rural population 12 locally grown food items had higher F' content (0.2 to 11 mg/Kg) with notable exception of coconut water that contained no F'.

2.2.3 Fluoride content of salt :

The table/cooking salt is also known to contain high F' levels. The consumption of salt is even greater in India due to hot climate (Sita and Venketeswarlu, 1967).

In India urban consumers go in for powdered packages brands of iodised and non-iodised salt. Majority of rural and significant proportion of urban population still purchase and use the raw unrefined salt after grinding it. In addition to table salt two other rock salt are used for flavouring special dishes like pickles and other savorites.

The rock salts (75% NaCl) have been known to be widely consumed as cooking salt in regions of the Punjab in India and contain F' in concentration as high as 200 ppm.

Gupta et al. (1991) reported that rock salts Kala Namak and Sendha Namak used in specially flavoured spicy dishes like pickles, chat etc. contained 2.75 and 2.50 ppm of F', respectively.

2.2.4 Fluoride content of tea :

Indian population consume substantial quantities of tea. Irrespective of socio-economic status, caste, creed and age group, the people are known to consume daily a few cups of excessively infused tea with sugar and milk. As tea leaves are known to contain concentrated amounts of aggravating factor in this country.

Tea leaves accumulate more F' than any other edible plant.

Nanda (1972) reported that two cups of tea provide 1 mg of F'.

Lakdawala and Punekar (1973) estimated the F' content of tea infusions was prepared by household methods. One cup of tea containing 100 ml infusion made from 1.0 g of tea sample yielded 0.3 mg F'.

Alary et al. (1977) estimated the F' content of beverages. Bottle vichy water had most F' 10.6 ug/g, tea infusion (2 g/100 ml) was 1.03 to 1.44, tea leaves 71.1 to 88.9 and milk 0.5 ug/g. Amountof F' in tea varied depending on type and quality. With extraction for a short time at 1%, tea bags provided most F' and coarse tea, showed the greatest proportional increase with long extraction.

Belbissi et al. (1988) studied the 2516 Jordanian children and found there was a positive correlation between the mean fluorosis index (4.07±0.10) and the daily mean numbers of cups of tea per child (5.84±0.69). They concluded that severity of dental fluorosis in children in Jordan may be related to excessive daily tea drinking which is the source of high and continuous F' uptake.

Wei. SH et al. (1989) estimated mean F' concentration in teas and found it to be 1.73 mg/kg in Chinese teas, 1.24 mg/kg in Ceylon/Indian teas. Herb teas contained a negligible amount of F' (0.02 to 0.05 mg/kg). The total F' content in tea leaves ranged from 82 to 371 mg/kg. The addition of milk to tea infusions did not appreciably reduce the F' concentration.

Gupta et al. (1991) analysed F' content of tea leaves from five popular brands varied from 20 to 165 ppm. Calculations made from the amount of F' ingested by a person consuming four cups (200 ml each) of tea daily ranged widely from 160 ug to 1320 ug.

Gupta et al. (1991) reported the F' content of infusion from different tea brands which ranged from 5 to 165 ppm in generating the cheaper varieties of tea leaves contained more F' as compared to costlier ones.

2.2.5 Fluoride content of toothpastes :

Toothpastes and mouth washes used personally and solutions applied in dental clinics may contribute significant amounts to overall F' intakes. Majority of the people in rural, urban and semi urban areas falling in fluorotic zones of India are known to use underground water for cooking and drinking purposes. Even as the Water Technology Mission of India is spending large sums of money on removal of excess F' and development of defluoridation plant and packages with a goal of not more than 1 ppm F' in drinking water, toothpaste manufactures continue to add F' in toothpastes and mouth washes indiscriminately.

Barnhart et al. (1974) studied the ingestion of F' dentrifice in 2-35 years olds and reported that 2-4 year old children ingested 35 per cent of the paste used for brushing. The per cent of paste ingested while brushing was 13.5, 6.4 and 2.9% in case of 5 - 7, 10-13 and 20-35 years olds, respectively indicating that younger the age, more is the risk of ingestion of F' dentrifice while brushing.

Davies and Winter (1977) reported that reduced dietary erosion of adult dentine and enamel by treatments with F' toothpaste slurry 1 minute before exposure to erosive agents (1% citric acid, orange juice etc.). They concluded that more damage occur to teeth by erosive agents than by toothpaste abrasion alone.

Burrell (1983) recommended that rinse should not be used by children under six years of age because control of their swallowing refluxes is not good.

Rajan et al. (1987) reported that use of F' containing toothpaste contribute to a grreat extent to the total intake of fluoride and leads to an increase in the serum and urine F' levels after using fluoride toothpaste.

Szpunar et al. (1988) reported that incidence of fluorosis increased with age and use of topical F' rinses. Results suggested that intake of F' from sources such as toothpaste, dietary supplements and F' extent of drinking water was the main source of differences in the incidence of caries and fluorosis.

Gupta et al. (1991) studied 14 brands of toothpaste and revealed F' content varied from 17.96 to 3169 ppm. Assuming that on an average 2 g of toothpaste is used by an individual, the amount in this portion varied from 0.02 to 4.25 mg. As per Eichler et al. (1955), 20% of F' is retained in a single brushing.

Keeping in view the very low F' requirements, the toothphastes which provide about 1 to 2 mg F' per brushing may add significantly amounts of F' to already critically high intakes of this element by the people.

2.3 Urinary excretion of fluoride :

The urinary F' level is widely used for the detection of endemic hydrofluorosis. It is excreted through the sweat and is found in traces in saliva, hair and tears but urine is the main excretory pathway. Therefore, of the amount ingested most absorption occurs passively from the stomach and 20-30 per cent of this is excreted in the urine in the next 3-4 hours (Carlon et al., 1960; Ericson, 1958; Smith, 1965).

Zipkin and Leone (1957) reported that F' intake is not directly correlated with age according to studies in which age groups of 7 through 16 years were compared to age groups of 20 years and over. Urinary excretion of F' is directly correlated with the level of F' intake. Approximately 60 per cent of F' ingested was excreted within the first 24 hours at various intake levels. When there was decrease in the amount consumed the urinary F' output decreased indicating that previously stored F' was being mobilized and excreted.

In a study by Toth and Sugar (1976) F' concentration in drinking water of four villages in Hungary was 0.40, 0.99, 1.44 and 1.52 ppm and average urinary F' excretion from 307 villagers was 0.57, 1.14, 1.23 and 1.58 ppm. They noted that excretion was greatest during the first eight hours and none of the subjects excreted the entire amount ingested within 24 hours.

Fluoride excretion in the urine is governed by glomerular filteration rate, urine flow rate and urine pH (Ekstrand et al., 1980) and also depends on total intake of F' , the form in which F' is taken into the body and exposure of the individual to the F' environment (Shivachandra and Thergaonkar, 1984).

Reddy et al. (1983) reported the range of minimum F' lvels in the urine to be 0.5 to 4.48 ppm and the maximum level to be 1.5 to 13 ppm. The F' concentration of well water ranged from 3.8 to 11.4 ppm. They noted wide fluctuations in urinary F' levels from day to day and no definite correlation was found between urinary F' levels and clinical stage and neurological sequelae.

Chandra and Thergaonkar (1983) reported that F' values in the drinking water varied from 1.42 ppm to 11.80 ppm while values of F' excreted in urine ranged from 0.8 to 30.4 ppm.

Mingxuan et al. (1984) reported a strong positive correlation between F' in urine and the amount of F' in drinking water. However, there was no significant differences between the patients and the normal persons.

Teotia et al. (1982) and Rajalakshmi (1984) reported urinary F' level to be misleading parameter. In a study, it was revealed that upto 30 days after

F' ingestion an increased amount of F' is excreted. Thereafter kidney tubules fail to function normally and the F' excretion gradually diminishes towards normal limits. The information available in human subjects affected with fluorosis do reveal wide variation in the urinary F' level. They concluded that severity of the disease and the urinary F' content have no correlation.

In another study by Teotia (1984) from the animal modal daily administration of 10 mg Na F/kg body weight in rabbits suggest that urinary F' level is neither a criterion to assess the severity of the disease nor to diagnose the disease.

Gupta (1988) reported that the prevalence rates and the severity of the disease run parallel to the concentration of F' in drinking water and there was strong positive correlation between the urinary F' level and the drinking water.

Kertisz (1989) reported a linear correlation between the 24 h urinary excreted fluoride quantity and F' creatinine ratio for the monitoring of urine samples of several children living in a children's home in Hungary. The mean values of F'/creatinine ratio in groups of children from and different parts of Hungary also showed a linear correlation with the F' concentration in drinking water. The urinary F' creatinine ratio give sufficient information for F' intake.

Chinoy et al. (1992) carried out a survey in 36 villages of Mehsana district of North Gujarat. Urine and blood samples of fluoride-afflicted human population and their drinking water were analysed for F' contents and compared with samples of Ahmedabad city (control). The F' content in water samples of Ahmedabad city was within the permissible limit, but was high in endemic villages. There is altered kidney formation in fluorosis afflicted individuals with high urine and serum F' levels.

CHAPTER-III

MATERIAL AND METHODS

The present study was conducted to determine the F' content in food, potable water and raw foods in village Gangwa near Hisar Town during three different seasons viz., summer, monsoon and winter. The comparison of F' intake and urinary excretion of the fluorotic and normal adults was undertaken. The detailed technical plan and the methods followed to obtain data on the lines of the objectives are given under the following sub headings :

3.1 Physiography of the area

3.2 Locale of research

3.3 Selection of village

3.4 Water sources survey and sample collection

3.5 Selection of respondents

3.6 Residential history, dental picture and other miscellaneous observations of the respondents

3.7 Collection of cooked food samples

3.8 Collection of raw food samples

3.9 Preparation of water, urine and food samples for analysis

3.10 Assessment of nutritional status of respondents

3.11 Fluoride estimation in water, urine and food samples

3.12 Statistical analysis

3.1 Physiography of the area :

Hisar district has been identified to the endemic for fluorosis in Haryana (Fig. 1). The district constitutes the western most extremity of Haryana and is the largest in the state. It lies on the confines of Rajasthan desert between 28°-59' south to 30° north latitude and 74°-32' west to 76°-18' east longitude. It is bounded by Bhiwani district and Jhunjhnu district (Rajasthan) in South, Rohtak and Jind district in its east, Bhatinda and Ferozepur districts (Punjab) in its north and Ganganagar and Churu districts (Rajasthan) in west . Soils are calcareous and usually have massive Kankar pan at depth of 0.75 to 1.25 m. Salinity and alkalinity are a serious problem. The maximum prevailing temperature is 45 - 48°C during summer and 2 - 3°C in winter. The average rainfall is 300 to 500 millimeters. The chief source of water supply for the population are the artificial wells of a very ancient type along with handpumps at certain places. Despite availability of piped water the people of rural area mainly drink underground water and are habitual consumers of spices, tea, tobacco and alcohol consumption is also common amongst people. The FNB surveys (1980) have revealed the Vitamin A, B and C group intake of the people of Hisar district to be much below the RDA's (ICMR, 1981). The average protein calorie, iron and calcium intakes were satisfactory.

3.2 Locale of research :

Hisar district of Haryana State was selected purposively for the sake of convenience of researcher.

3.3 Selection of village :

A preliminary survey was done for prevalence of dental fluorosis in 8 randomly selected villages from amongst the villages which were within 15 KM radius of Hisar city. The population of all these 8 villages were seen to be affected by dental fluorosis. Out of these one village 'Gangwa' which had substantial number of adults with dental mottling was selected for the purpose of further studies.

3.4 Water sources survey and sample collection :

A preliminary survey of housing clusters and sources of water used for drinking and cooking purpose by the population of Gangwa village was made. The people within the village lived in different Mohallas. Each Mohalla had particular caste domination. The village had predominantly more Kumhars followed by Jat, Chamars, Bishnoi and Pandit families. The village had 4 wells and 4 handpumps and 10 municipal taps in all. With regards to the location, each of the four wells were situated in different Mohalla. Out of four handpumps, two were within the compounds of two houses in Kumhar's Mohalla. Remaining two handpumps were situated at two common places but were within the reach of the Chamars' Mohalla. The handpumps were known to contain sweet water by villagers. Majority of the people who could borrow or draw water from these four handpumps did so to use it for drinking and cooking purposes. In comparison to municipal water the wells and handpumps were considered to be better than tap water for consumption because of taste preference.

Water sample collection :

The water samples from 8 ground water sources and one tap water were collected during the year 1992 in three different seasons viz., summer, monsoon and winter. The collection for winter season was during November, for summer in May and for monsoon in August. Water samples were drawn in the morning in polythene bottles and taken to laboratory for estimation of F'.

3.5 Selection of respondents :

It was observed that the habitation of people was divided in different Mohallas wherein the people's residence was predominantly caste based. Each Mohalla had different ground water source. Therefore, people belonging to different castes used water from different water sources. All the adult residents (18 to 45 years) of Mohallas were examined for dential fluorosis prevalence using a proforma prepared by Gupta (1988) (Appendix-I). Thereafter randomly 25 male and 25 female fluorotic adults and similar number of normal adults were selected. During the selection care was taken to choose respondents using the water from the ground water-sources as well as municipal water.

3.6 Residential history, dental picture and other miscellaneous observations of the respondents :

A questionnaire was prepared and pretested on 10 per cent of the population to be actually studied. These respondents were excluded in the actual sampling. Necessary modifications were made in the questionnaire. The questionnaire included questions of general information including the age, caste, education, type and size of family, residence history, water used for cooking and drinking, commonly used green leafy vegetables, spice consumption trends, brands, method of preparation and frequency or quantity of tea consumption, cereal milling techniques. The fluorotic respondents were observed for the degree of dental fluorosis as per the Deans (1934) classification. The questionnaire is given in Appendix-II.

3.7 Collection of cooked food samples :

The weighment of cooked method (ICMR, 1951) was used. This proforma was modified to include information on the three seasons for an individual subject. The additional questions were included to elicit information on the number of glasses/amount of drinking water and tea consumed. The total food and fluids consumed during each meal by the each subject was recorded and a one-fifth portion of his/her total intake was collected in a polythene bag or bottles personally by the researcher. Drinking water consumed during 24 hours period was recorded for each subject. The subjects were requested to remember the number of glasses of water consumed during the 24 hours period and also of other liquids like buttermilk, milk and tea and any other beverages consumed by them. Records of these were carefully made by the researcher daily. All these observations and collections were repeated for the same 100 adults for three seasons viz., summer, monsoon and winter. The food weighing balance and measuring cups-spoons, were used by the researcher in each household studied.

3.8 Collection of raw food samples :

On the basis of the preliminary food consumption of the families a list of all the perishable and non-perishable foods consumed by the subjects during three seasons were prepared. The source of each food item was also ascertained. During the three seasons the samples of food actually being consumed by the respondents were collected. These included the cereals whole and powdered, pulses whole, split or dehusked including Basen; vegetables, spices, tea etc.. The roti samples were also collected. The cereal and pulse grinding techniques of the families were also recorded.

3.9 Preparation of water, urine and food samples for analysis :

The 100 ml polythene bottles were dipped in 2.5 N HCl for 12 h and then thoroughly washed with teepol and finally with double distilled water, dried and labelled. Maximum care was taken to avoid contamination of bottles with dust particles. After rinsing the bottles about 100 ml of water was collected for the analysis of F'. Spot urine samples of all the 100 subjects were collected for all the three seasons. The food samples brought to the laboratory for each subject for each day was pooled, and homogenised in a mixer-cum-blender (with stainless blades). A suitable aliquot of sample was dried in an electric oven at a temperature of about 70°C. The dried sample was powdered in cyclotec mill (60 mesh sieve) and stored in polythene bottles and used for analysis of F' content.

3.10 Assessment of nutritional status of respondents :

Nutritional status may be defined as condition of health and is influenced by the intake and utilization of nutrients (Caliendo, 1979). In the present study the heights and weights were recorded for the nutritional status assessment of each subject, details of which are given below :

Height (Ht.) :

Jelliffe (1966) reported that height is affected only by long term nutritional deprivation and considered an index of chronic and long duration malnutrition.

Height was measured according to the method described byJelliffe (1966). A vertical measuring rod caliberated in centimeters made by Western surgical manufacturers was used. The bare footed respondent was made to stand erect on platform with feet parallel with heels, buttocks, shoulders and back of head touching the upright. The head was held comfortably erect, with the lower border of the orbit in the same horizontal plane. The arms were hanging at the sides in a natural manner. The head piece was gently lowered, crushing the hair, making contact with the top of the head. Height measurement was repeated for each individual and then mean was taken.

Weight (Wt.) :

It is good indicator of present nutritional status and is concerned with determining degrees of under-nutrition principally resulting from varying levels of protein calorie malnutrition. It is also concerned with the detection of obesity (Jelliffe, 1966).

Indian made weighing balance (Krups) caliberated in kilograms and grams was used for weighing by method described by Jelliffe (1966). The respondent was made to stand bare footed on the platform of balance without touching any other surface or subject with minimum clothes. The pointer on balance scale was adjusted to zero before each weighing. It was repeated three times for each individual and then mean was taken.

3.11 Fluoride estimation in water, urine and food samples :

(i) Fluoride estimation in water and urine :

Fluoride in water and urine was determined by Spectrophotometric method using Zirconium - Eriochrome - Cyanine R Lake (Megregian, 1954).

Preparation of stock solution :

1. Stock Solution, 1000 ppm : Dissolved 2.211 g of analytical grade sodium fluoride (dried at 110°C for 2 h) in double distilled water and diluted to 1 litre and stored in plastic bottles.
2. Intermediate Standard Solution, 100 ppm : Took 10 ml of the stock solution and diluted to 1 litre and stored in plastic bottles.
3. Working Standard Solution, 10 ppm : Took 10 ml of the intermediate solution and diluted to 1 litre and stored in plastic bottles.
4. Hydrochloric acid, 7:3 HCl Distilled water.
5. Ziroconium - Oxychloride Solution : Took 0.265 g and dissolved in about 5 ml of distilled water. The volume was made up to 1 litre with 7:3 HCl.
6. Eriochrome Cyanine R-Lake Solution : 1.8 g of eriochrome cyanine was dissolved in double distilled water and diluted to 1 litre.
7. P-nitrophenol - 1 g of P-nitrophenol was dissolved in double distilled water and diluted to 1 litre.

Preparation of sample for tea leaves and salt :

Tea leaves extraction :

1 g of tea leaves were boiled in 100 ml of tap water in a 250 ml beaker (uncovered) and boiled for 5 minutes. The tea leaves were allowed to settle at the bottom of the beaker and tea extract poured out in another

beaker. Added one and a half tea spoon of activated charcoal. Allowed to stand for 5 minutes and filtered through Whatman No. 42 filter paper and stored in plastic bottles.

Salt sample :

To 1 g of crude.salt (dried at 100°C for 12 hours) and dissolved in 100 ml of distilled water and then made the volume to 100 ml and stored in plastic bottles.

Procedure :

1. Maximum blank : Pipetted out 5 ml of double distilled water in a 50 ml volumetric flask. Added a drop of p-introphenol indicator. If yellow colour appears, then added 7:3 HCl dropwise in which yellow colour disappeared. Washed the necks of the flask with a jet of double distilled water. Then added 5 ml of Zirconium oxychloride solution to the flask. Again washed the necks of the flasks and added 5 ml of Eriochrome cyanine R solution. Made the volume to the mark with double distilled water. Shook well. Kept for 5 minutes for full colour development. Read the transmittance at 527.5 nm, wavelength.
2. 100 per cent transmittance setting : Repeated the same procedure as in maximum blank except that 5 ml of 7:3 HCl was taken in place of Zirconium oxychloride solution.
3. The standard curve was prepared by taking 0.5, 1, 2, 3, 4, 5, 6 ml of 10 ppm F' working standard. Repeated the same procedure as in maximum blank.

Similarly depending on the fluoride content of the sample, 0.1 to 10 ml of the sample solution was taken and further proceeded as for the maximum blank.

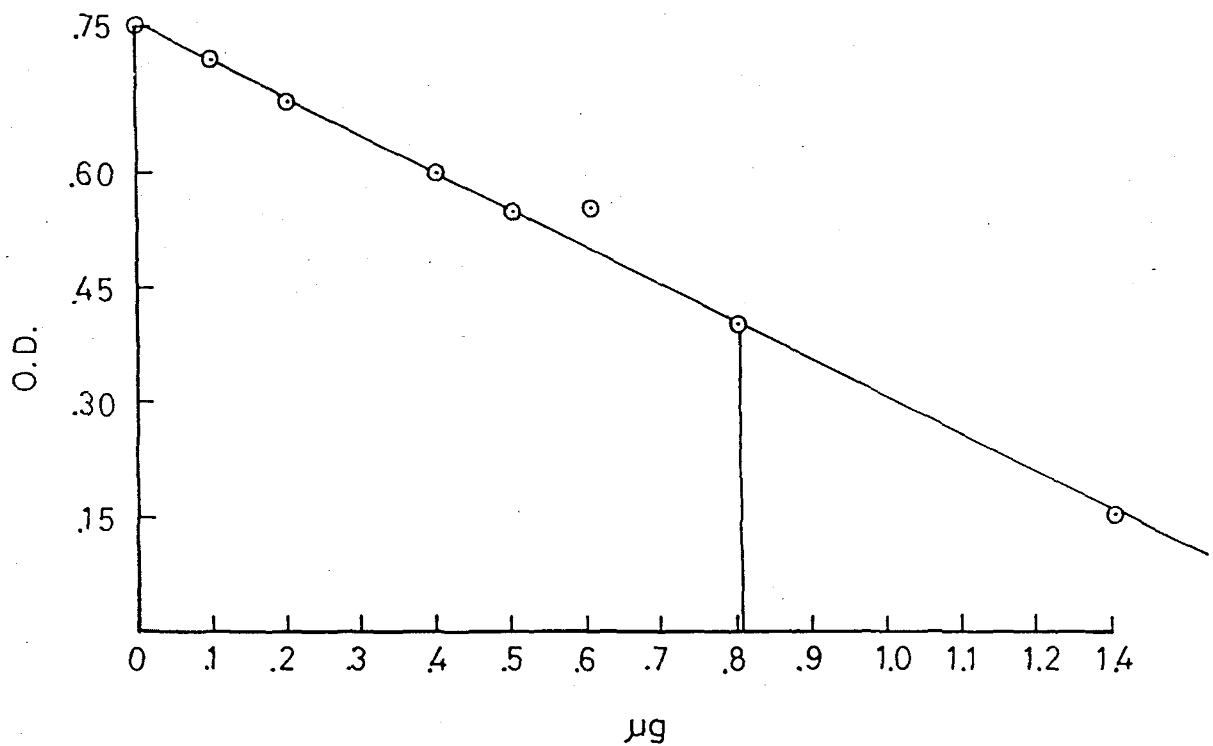

FIG. 2. STANDARD CURVE FOR FLUORINE (ppm F)

(ii) Fluoride estimation :

Fluorine in food samples was determined by the method of Villa (1979) using an ion-selective electrode.

Reagents :

1. Fluoride standard solution 2000 ppm : Dissolved 4.421 g of analytical reagent grade sodium fluoride (dried at 110°C for 2 hours) in double distilled water and diluted to 1 litre.
2. Perchloric acid, 0.1 N : Diluted 10.3 ml of 62 per cent analytical reagent grade perchloric acid (specific gravity 1.52) to 1 litre with double distilled water.

Procedure :

Dried the sample for 24-48 hours at 80°C and ground it to pass through a 60 mesh sieve and stored in clean, dry, air tight plastic containers. Weighed 1.0 of powdered sample and placed it in a 100 ml plastic beaker. Added 25 ml of 0.1 N perchloric acid and stirred magnetically for 20 minutes. Added an additional 25 ml of 0.1 N perchloric acid and stirred magnetically for 5 minutes. Inserted the Fluoride specific ion electrode - 9040900 and Reference electrode - 900100 while continuing to stir. For blank took 50 ml of perchloric acid in a beaker.

Took the reading (E_1) of blank when stablize (not more than 5 minutes) on Systonic Digital pH meter. Added 0.2 ml of the Fluoride Standard Solution (2000 ppm) and noted the reading (E_2). Similarly took the reading of the sample. Determined the F' concentration in the sample using an equation given below :

Calculations :

$$\text{Concentration of fluorine } (C_F) = \frac{Ma}{m(\log^{-1}(\Delta E/S) - 1)}$$

C_F = Total F' concentration in mg/g or ppm

Ma = Mass of fluoride added.

m = Dry mass of samples in grams

ΔE = The difference between E_1 and E_2

ΔE = E_1-E_2. (E_1 = Observed potential in mv; E_2 = Potential observed after adding 0.2 ml of 2000 ppm F' solution)

S = Electrode slope i.e. 58.5 mv.

3.12 Statistical analysis :

The data were laid down in complete block randomized design and statistically analysed for analysis of variance according to Henry (1970). The data were also analysed for coefficient of correlation.

CHAPTER-IV

RESULTS AND DISCUSSION

Fluoride content was determined in 9 underground water sources, 300 spot urine samples, 300 composite diets for the three different seasons. Suitable number of raw foods (perishable and non-perishable) mainly consumed by the rural population were also analysed for F'. The results obtained have been expressed under the following sub-headings :

4.1 Mean underground potable water F' level of village Gangwa in different seasons;

4.2 Sex wise distribution of the selected fluorotic and normal adults;

4.3 Sources and F' levels of drinking water used by fluorotics and normal rural adults;

4.4 Distribution of the normal and fluorotic subjects according to their drinking water F' levels;

4.5 Degree of dental fluorosis and drinking water F' levels of fluorotic subjects;

4.6 Degree of dental fluorosis in the fluorotic adults on the basis of age;

4.7 Distribution of the normal and fluorotic adults according to their nutritional status;

4.8 Fluoride content of raw vegetables;

4.9 Fluoride content of common pulses and legumes;

4.10 Fluoride content of raw and cooked cereals;

4.11 Fluoride content of common spices and condiments;

4.12 Fluoride content of miscellaneous items;

4.13 Seasonal variation in daily F' intake from drinking water;

4.14 Seasonal variation in mean daily F' intake from food excluding tea of fluorotic and normal rural adults;

4.15 Seasonal variation in daily F' consumption through tea of fluorotic and normal rural adults;

4.1 Mean underground potable water F' levels of village Gangwa in different seasons :

The data on potable water F' levels from municipal supply, wells and handpumps along with their approximate depths during summer, monsoon and winter are shown in Table-1.

The water F' levels from all the 3 sources ranged from 1.03 to 4.53 ppm. In the municipal water mean F' values were 1.13 ppm in summer, 1.07 ppm in winter and 1.03 ppm in monsoon season. The summer and winter values were similar and monsoon and winter values were similar. However, summer values were significantly (P/ 0.05) higher than monsoon values.

Well waters were compared with each other for their depth and the F' content during the different seasons. There were four wells, the depths of which were 18, 19, 21 and 22 feet. The water from all these four wells contained F' from 2.03 to 3.07 ppm F'. The wells with depths 18, 19, 21 and 22 feet were found with water F' levels of 3.07, 2.40, 2.03 and 2.33 ppm during summer season. The maximum value was 3.07 ppm at 18 feet and minimum was 2.03 at 21 feet. During this season all the values of well waters varied significantly (P / 0.05) when compared with each other. The well waters in monsoon season contained significantly (P / 0.05) higher amount of F' in comparison to that in both summer and winter season.

Table 1 : Mean underground potable water F' levels of village Gangwa in different seasons

Water sources	Depth (ft.)	F' content (ppm)		
		Summer	Monsoon	Winter
1. Municipal water	-	1.13±0.13	1.03±0.03	1.07±0.07
2. Wells				
W_1	22	2.33±0.07	3.07±0.07	2.73±0.07
W_2	21	2.03±0.03	2.33±0.07	2.20±0.00
W_3	19	2.40±0.00	2.33±0.07	2.23±0.09
W_4	18	3.07±0.07	3.57±0.07	3.13±0.07
3. Handpump				
H_1	18	4.03±0.03	4.10±0.06	4.07±0.07
H_2	18	4.33±0.07	4.53±0.07	4.07±0.07
H_3	20	2.03±0.03	2.33±0.07	2.07±0.07
H_4	21	3.07±0.07	3.33±0.07	3.03±0.07

CD at 5% level = 0.09.

The handpump waters when compared with each other for their depth and the F' contents during different seasons showed that there were four handpumps with 18 to 21 feet depth. During monsoon season, the handpump water F' content was significantly (P / 0.05) higher in comparison to summer and winter. The water F' content of handpumps at 18 feet depth had varying F' content and all were located at different places in the village. Therefore, the differences could be attributed to the locational variation. Locational variation in F' content of water from same depth may be due to irregular distribution of F' bearing minerals in soil which are main sources of F'. Similar results were reported by Singh et al. (1962), Anand et al. (1963) and Ramamohana and Bhaskaran (1964). Anand et al. (1963) observed that in the same village (Bindupur, near Delhi) the F' content of wells varies from 0.40 to 4.00 ppm. Mehta (1960) reported the F' content of 18 well water samples of Hisar and Sangrur districts to range between 0.11 to 13.64 ppm. Similarly, Susheela (1984) also estimated the water F' levels to be 0.40 to 8.00 ppm from wells, tubewells and ponds in Amerli district of Gujarat. Gupta (1988) observed F' content of underground water samples collected from all over Hisar to range between 0.05 to 14.6 ppm. Mehta (1960) found the well water F' content to be increased with corresponding increase in depth. However, Ramamohan and Bhaskaran (1964) observed no correlation between F' content and the depth of water. Nanda and Kapoor (1972) found the F' content of 100 well water samples to be significantly higher in monsoon as compared to summer and winter season. However, Ray et al. (1983) observed no significant differences in F' content of well water samples during summer and post rainy season.

4.2 Sex wise distribution of the selected fluorotic and normal adults :

From data in Table-2 it is evident that out of a total population of 100 selected rural adults 13% were in the age group 20-25 years, 19% in the category of 25-30 years and 18% in the age group of 40-45 years. About 25% each belonged to the age group of 30-35 and 35-40 years, respectively. Thus, it can be concluded that the maximum number of selected respondents aged between 30-40 years and that each age group was represented.

4.3 Sources and F' levels of drinking water of fluorotic and normal rural adults :

Table-3 indicates that out of a total population of 100 rural adults 6% of the subjects consumed water with a mean F' content of 1.08 ppm through municipal water. The well water mean F' content of 2.19 - 3.26 ppm ranged for all the seasons. The percentage of subjects drinking water of the W_1 well (2.71 ppm F') were maximum (30%) followed by W_4 (3.24 ppm F') were 10%, W_2 (2.19 ppm F') were 9% and W_3 (2.34 ppm F') were 11%, respectively.

The F' content of handpumps as mean values of three seasons ranged from 2.23 - 4.31 ppm. The number of subjects using water from H_4 (3.24 ppm F') were maximum followed by those from H_1 (4.07 ppm F'), H_3 (2.23 ppm F') and least number were water from H_2 (4.31 ppm F').

4.4 Distribution of the normal and fluorotic subjects according to their drinking water F' levels :

Table-4 indicates that maximum (30%) of the selected subjects used water containing 2.5 - 3.0 ppm F' for cooking and drinking purposes. A little lesser (28%) number of subjects reportedly consumed water with 2 - 2.5 ppm F'. Still lesser but substantial number of subjects consumed water with 3.0 to 3.5 ppm F'. Nine per cent and 6% subjects consumed water 3.5 - 4 ppm and $>$ 4.0 ppm F', respectively. The number of fluorotics and the normals were compared in each

Table 2 : Sex-wise distribution of the selected fluorotic and normal adults

Sr. No.	Age group (Years)	Subjects				Total (%)
		Fluorotic		Normal		
		Male	Female	Male	Female	
1.	20 - 25	4	2	4	3	13
2.	25 - 30	3	5	5	6	19
3.	30 - 35	5	9	6	5	25
4.	35 - 40	7	4	6	8	25
5	40 - 45	6	5	4	3	18
	Total	25	25	25	25	100

Table 3 : Sources and F' level of drinking water of fluorotic and normal rural adults

Water sources	Water F' (ppm)	Total subjects	Subjects					
			Normal			Fluorotic		
			Male	Female	Total	Male	Female	Total
1. Municipal water	1.08	6	2	2	4	1	1	2
2. Wells								
W_1	2.71	30	7	8	15	6	9	15
W_2	2.19	10	3	1	4	4	2	6
W_3	2.34	9	2	2	4	2	3	5
W_4	3.26	11	3	4	7	2	2	4
3. Handpump								
H_1	4.07	9	2	1	3	4	2	6
H_2	4.31	6	1	2	3	2	1	3
H_3	2.23	9	3	2	5	1	3	4
H_4	3.24	10	2	3	5	3	2	5

* Mean of values in three seasons (summer, winter and monsoon).

Table 4 : Distribution of the normal and fluorotic subjects according to their drinking water F' levels

Drinking water	Subjects		Total
	Normal	Fluorotic	
1.08	4(66%)	2(33%)	6
2-2.5	13(47%)	15(53%)	28
1.5-3.0	15(50%)	15(50%)	30
3.0-3.5	12(57%)	9(43%)	21
3.5-4.0	3(33%)	6(66%)	9
> 4.0	3(50%)	3(50%)	6
Total	50	50	100

water F' level category. It was observed that except in the 1.08 ppm and 3.5 to 4.0 ppm category of water F', the number of fluorotic and non-fluorotic subjects were almost equal in all other categories. The exception was seen in the 3.5 - 4.0 ppm F' category where the ratio of non-fluorotic to fluorotic adults was 33:66. In the second exception of 1.08 ppm F' category ratio of non-fluorotic to fluorotic subjects was 66:33. Therefore, the number of fluorotic subjects from 1.08 water F' category are the least. All other water F' categories had about 50% of subjects as fluorotics.

4.5 Degree of dental fluorosis and drinking water F' levels of fluorotic subjects :

Table-5 represents out of the 50 fluorotics 2% (questionable), 10% (very mild), 26% (mild) and 62% moderate cases were observed. There was not a single case of severe grade as defined by Dean (1934). Observations of the degrees of dental fluorosis revealed out the total fluorotic subjects studied 4% consumed municipal water with mean 1.08 ppm F' level. An equal number (30% each) of fluorotic subjects were between categories at 2 - 2.5 and 2.5 - 3.0 water F' levels. The number of fluorotic subjects drinking water with 3.0 - 3.5, 3.5 - 4.0 and $>$ 4 ppm was 18%, 12% and 6%, respectively. Out of the total 2 subjects consuming water with 1.08 ppm water F', one had questionable and other had the mild degree of dental fluorosis. Of the subjects using water with 2 - 2.5 ppm F' levels, 6.67%, 20%, 73.34% had very mild, mild and moderate degree of dental fluorosis, respectively. Of the fluorotic subjects in the 2.5 - 3.0 ppm F' category 13% had very mild, 33.3% had mild and 54% had moderate degree of dental fluorosis. The degree of dental fluorosis at water F' level of 3.0 - 3.5 ppm F' was very mild in 22%

Table 5 : Degree of dental fluorosis and drinking water F' levels of fluorotic subjects

Drinking water	Degree of dental fluorosis				Total
	Questionable	Very mild	Mild	Moderate	
1.08	1(50%)	0	1(50%)	0	2(4%)
2-2.5	0	1(6%)	3(20%)	11(74%)	15(30%)
2.5-3.0	0	2(13%)	5(33%)	8(54%)	15(30%)
3.0-3.5	0	2(22%)	3(33%)	4(45%)	9(18%)
3.5-4.0	0	0	1(16%)	5(84%)	6(12%)
> 4.0	0	0	0	3(100%)	3(6%)
	1(2%)	5(10%)	13(26%)	31(62%)	

mild in 33% and moderate in 45% subjects. At water F' level 3.5 - 4.0 ppm, maximum (84%) of the subjectshad moderate degree of dental fluorosis and 16% of the subjects with mild degree were observed. All the subjects had moderate degree of dental fluorosis at 4.0 ppm F' level and no case of other degree was reported.

4.6 Degree of dental fluorosis in fluorotic adults on the basis of age :

Prevalence of different grades of dental fluorosis was as follows :

Questionable - 12%; very mild - 10%; mild -26% and moderate -52%, respectively. Maximum number of subjects had moderate degree of dental fluorosis.

Out of the total selected fluorotics, 12% were in 20-25 years age group, 16% in 25-30 years age group and 28% in the 30-35 years age category. 22% subjects of each were 35-40 years and 40-45 years old. In the 20-25 years olds, 4% had questionable, 2% had mild and 6% moderate degree of dental fluorosis. In the 25-30 years old group, fluorotic subjects 10% had moderate and 6% had mild degree of dental fluorosis. No subject in this age group had questionable or mild degree of dental fluorosis. Out of the 30-35 years age group 14% had moderate, 6% had mild and 8% very mild degree of dental fluorosis. No case of questionable degree was observed during this age group. Out of 35 -40 years old fluorotics, 8% each were in the mild and moderate and 6% had questionable degree of fluorosis. Out of 40 - 45 years old fluorotic subjects 2% had questionable 2% had very mild, 4% mild and 14% moderate degree of dental fluorosis (Table-6).

Bagga _et al._ (1979) observed that dental fluorosis was more in persons over 15 years than among those below 15 years whereas Short _et al._ (1937) reported that prevalence of dental fluorosis beyond 20 years gradually declined.

Table 6 : Degree* of dental fluorosis in the selected rural fluorotic adults on the basis of age

Sr. No.	Age group (Yrs.)	Degree of dental fluorosis				Total
		Questionable	Very mild	Mild	Moderate	
1.	20-25	2(4%)	0	1(2%)	3(6%)	6(12%)
2.	25-30	0	0	3(6%)	5(10%)	8(16%)
3.	30-35	0	4(8%)	3(6%)	7(14%)	14(28%)
4.	35-40	3(6%)	0	4(8%)	4(8%)	11(22%)
5.	40-45	1(2%)	1(2%)	2(4%)	7(14%)	11(22%)
	Total	6(12%)	5(10%)	13(26%)	26(52%)	50(100%)

* *Dean's (1934) classification*

Fig. 3 Mild degree of Dental fluorosis in age group of 30-35 years.

Fig. 4 Moderate degree of Dental Fluorosis in age group 30-35 years.

Daves (1945) also concluded that chalkiness might disappear after 30 years of age.

4.7 Distribution of the normal and fluorotic adults according to the nutritional status :

Out of the 25 fluorotic males maximum number of subjects were found to be mildly and severely undernourished. The percentage of subjects in these two categories of undernutrition was 36% and 32%, respectively. Twenty per cent of the male fluorotics were having normal nutritional status and the 8% were overweight and 4% obese. When the non-fluorotic males were observed for their nutritional status the maximum number (36%) had mild undernutrition. Severe undernutrition was found in 16% normal males which are about less than half of that found in fluorotic males. More (28%) non-fluorotic subjects were found to be normally nourished than fluorotic subjects the corresponding figure for which was 20%. Similarly, there were double number (16%) of the normal overweight males than the fluorotic overweight males (8%). Obesity was found in least (4%) and equal number of fluorotic and normal males.

The trend of prevalence of severe undernutrition in the selected fluorotic and normal females was similar to that found in males i.e. double number severely undernourished females were fluorotic than the number of normal females. However, more proportion of females both fluorotic and non-fluorotic were there in the severely undernourished group as compared with number of fluorotic and normal males. Further equal number of females (20%) were found to be mildly undernourished. The number of mildly undernourished females was significantly less than the total number of both fluorotic and normal males. The normal females were 3 times more than fluorotic females with normal

Table 7 : Distribution of the normal and fluorotic adults according to their nutritional status

Grade of malnutrition	Critical limits of weight for height %	Subjects						Grand Total
		Male			Female			
		Fluorotic	Normal	Total	Fluorotic	Normal	Total	
Severe undernutrition	60	9(36%)	4(16%)	13	12(48%)	6(24%)	18	31
Mild undernutrition	60-80	8(32%)	9(36%)	17	5(20%)	5(20%)	10	27
Normal	80-110	5(20%)	7(28%)	12	3(12%)	9(36%)	12	24
Overweight	110-120	2(8%)	4(16%)	6	5(20%)	3(12%)	8	14
Obesity	-120	1(4%)	1(4%)	2	0(0%)	2(8%)	2	4

nutritional status. When these figures of normal nutritional status were compared sexwise it was seen there were more 36% non-fluorotic females and 28% non-fluorotic males, but the number of fluorotic males (20%) was more than the fluorotic females (12%). In the overweight category 16% normal and 8% fluorotic males were there and 12% normal and 20% fluorotic females were seen. Obesity was found in 4% each of the fluorotic and normal males. None of the fluorotic females was obese and 8% of the normal females were found to be obese (Table-7).

Shukla (1982) reported that diets inadequate in calcium, ascorbic acid or protein that is the poor nutritional status make the rural population more prone to fluorosis as compared to the well nourished counterparts on the same levels of fluoride intake.

4.8 **Fluoride intake of raw vegetables :**

On fresh matter basis the F' content of potato was maximum (0.45 ppm) followed by carrots (0.44 ppm) and onion (0.44 ppm), respectively. However, ginger (0.08 ppm) and radish (0.04 ppm) had minimum F' content. Among all the green leafy vegetables, chana leaves (0.36 ppm), spinach (2.6 ppm) were notably high F', while other vegetables had F' content ranging from 0.05 to 0.1 ppm. It was further revealed that dried vegetables had higher F' content as compared to the fresh ones. The F' content of roots and tubers is given in Table-8. On dry matter basis the F' content of carrots, onion and potato was 3.2, 2.5 and 1.8 ppm, respectively which was notably high than radish (0.08 ppm) and ginger (0.04 ppm). Among moisture free green leafy vegetables spinach, chana and mint leaves contained 3.8, 1.8 and 0.5 ppm F', respectively. Other vegetables like red chillies contained maximum (10 ppm) F' content. Similar results were reported by Nanda and Kapoor (1972) and Lakdawala and Punekar (1972). Wakode

Table 8 : Fluoride content of raw vegetables

		Name of the vegetable	F' (ppm) (on dry matter basis)	F' (ppm) (fresh matter basis)
(a)	Roots and tubers			
	1.	Potato	1.8	0.45
	2.	Onion	2.5	0.40
	3.	Carrots (red)	3.2	0.44
	4.	Radish (whole)	0.8	0.04
	5.	Ginger	0.4	0.08
(b)	Green leafy vegetables			
	1.	Spinach leaves	3.8	0.26
	2.	Chana leaves	1.8	0.36
	3.	Mint leaves (fresh)	0.5	0.05
(c)	Other vegetables			
	1.	Tomato	1.2	0.08
	2.	Cabbage	1.2	0.18
	3.	Peas green	1.0	0.18
	4.	Calabash cucumber (Lowki)	0.4	0.16
	5.	Cauliflower	1.0	0.01
	6.	Bitter gourd	0.3	0.024
	7.	Green chillies	1.5	0.12
	8.	Red chillies	10.0	1.05
	9.	Tinda	1.0	0.065
	10.	Lobia ki phali	1.0	0.15

(1990) in a study of endemic fluorotic area in Maharashtra reportec notably high F' content of red gram (11.20 ppm), red chillies (10.9 ppm), beans (6.90 ppm), jowar (6.2 ppm) and brinjal (3.90 ppm). Gupta (1991) reported the F' content of green leafy vegetables spinach, cabbage and chulai to be 4.6, 1.64 and 17.02, respectively.

4.9 Fluoride content of common pulses and legumes :

Bengal gram (whole) and bengal gram flour contained maximum 4.5 and 6.2 ppm F' content, respectively. Moth beans had 2.8 ppm, bengal gram Kabuli (whole) had 2.8 ppm, green gram (whole) had 2.7 ppm and black gram (whole) had 0.8 ppm F', lentil whole (1.0 ppm) and Red gram (whole) had(1.2 ppm). The Bengal gram is commonly used by the rural population due to their own production of this pulse. Higher levels of F' have also been reported in Bengal gram by Nanda and Gitonga (1983) in a study of North India (Lucknow). The F' content of Bengal gram was maximum (3 ppm) and the uncooked vegetables, fruits and fats were not rich sources of F' according to their study. The milled and dehusked legumes contained higher amounts of F' as compared to whole ones. Besan had maximum (6.2 ppm) and black gram (dehusked) had 1.2 ppm and green gram dehusked had 3.0 ppm of F' (Table-9). Similar results were reported by Lakdawala and Punekar (1973) in a study of Bombay (India) that milling procedures increase F' concentrations in wheat and bengal gram flours. This may be attributed to the milling procedure by the contamination through local grinder made of corrundom stone which contains high F' compounds.

4.10 Fluoride content of raw and cooked cereals :

Raw bajra, wheat and rice contained 1.8, 0.8 and 0.7 ppm F' level, respectively. The <u>Roti</u> of bajra and wheat contained 2.2 and 1.5 ppm F'.

Table 9 : Fluoride content of common pulses and legumes

Sr. No.	Legumes	F' (ppm)
1.	Bengal gram (whole)	4.5
2.	Bengal gram Kabuli (whole)	2.8
3.	Besan	6.2
4.	Lentils (whole	1.0
5.	Red gram (whole)	1.2
6.	Black gram (whole)	0.8
7.	Black gram (dehusked)	1.2
8.	Green gram (whole)	2.7
9.	Green gram dehusked	3.0
10.	Moth beans	2.8

Bajra khicheri contained maximum F' content 3.0 ppm due to salt, spices and wter being used in preparation (Table-10). Similarly the wheat and bajra roti may have contained higher F' than their respective flours due to F' added through water and grinding procedures. Similar results were reported by Nanda and Gitonga (1983) that high F' content in cooked foods was due to the water, salt and spices used in cooking.

4.11 Fluoride content of common spices and condiments :

The different spices being commonly used by the selected families are listed in Table-11. The F' content of cardamom big (12.5 ppm), red pepper (10.2 ppm) and garam masala (8.4 ppm) and coriander powder (8.0 ppm) were notably high. Turmeric powder, cumin seeds, fenugreek seeds and pepper also contribute significant amount of F' to the diets. Nanda and Gitonga (1983) reported F' content of cardomom big (12 ppm) and red pepper (10.0 ppm) to be higher than other spices and condiments . Although the spices are known to contain such high amounts of F'. Their contribution of F' to the diets of people under survey may be limited as majority of the people eat their flat bread with just salt, red pepper and onion. The spices are expensive food commodities and not essential ingredients. So people use them sparingly, if not within reach.

4.12 Fluoride content of miscellaneous items :

The two kinds of salt being used by people are unbranded (Dali ka Namak) and iodised refined salt. The F' content of unbranded salt and iodised salt was found to be 2.5 and 1.2 ppm, respectively. Gupta (1991) reported the F' content to range from 0.25 to 3 ppm, The sample of unground (rock salt) contained 3 ppm and Tata iodised salt, 1 ppm F'. Salt is consumed in large quantities in India as reported by Sita and

Table 10 : F' content of raw and cooked cereals

Sr.No.	Sample	F' (ppm)
1.	Bajra (raw)	1.8
2.	Bajra Roti	2.2
3.	Bajra Khicheri	3.0
4.	Wheat (raw)	0.8
5.	Wheat Roti	1.5
6.	Rice (raw)	0.7

Table 11 : Fluoride content of common spices and condiments

Sr.No.	Item	F' (ppm)
1.	Turmeric powder	4.2
2.	Cumin seeds	3.5
3.	Fenugreek seeds	3.9
4.	Coriander seeds (powder)	8.0
5.	Ajwain	3.2
6.	Garlic (dry)	0.83
7.	Pepper	3.5
8.	Red chilli powder	10.2
9.	Garam masala	8.4
10.	Cardamom big (whole)	12.4
11.	Cardamom small (whole)	7.3
12.	Sonf	4.0
13.	Cloves	3.0

Table 12 : Fluoride content of miscellaneous items

Sr.No.	Sample	F' (ppm)
1.	Sugar	0.3
2.	Tea (unbranded)	30
3.	Tea infusion	0.3
4.	Tata iodised salt	1.2
5.	Unbranded salt (Rock salt)	2.5

* 1 g boiled 5 min. in 100 ml. water.

Venketeswarlu (1967). Only unbranded salts were used by the people. Lakdawala and Punekar (1973) and Nanda and Kapoor (1972) observed that the unbranded tea samples had 30 ppm of F'. Generally the cheaper varieties of tea leaves contain more F' as compared to the superior ones due to the fact that mature leaves are used for their preparation. Tea contributes a valuable amount to daily F' intake. A 100 ml infusion made from 1.0 g of tea sample after 5 minutes boiling yielded an average of about 0.3 mg F'. Similar results were obtained by Lakdawala and Punekar (1973). Nanda (1972) reported that two cups of tea provide 1 mg of F'. Gupta (1991) observed that the F' content of various brands of tea infusion ranged from 5 to 165 ppm.

4.13 Seasonal variation in daily F' intake from drinking water :

The data of F' intake from drinking water are presented in Table-13.

The mean F' intake (mg/day) varied from 0.74 in case of normal females during winter season to 6.16 in fluorotic males during summer season through drinking water.

The seasons significantly affected the F' intake as detailed below :

Summer :

The mean F' intake of 6.16 and 4.87 mg/day of the male and female fluorotic subjects were significantly ($P < 0.05$) higher than the mean value of 5.35 and 3.86 mg/day of their normal counterparts, respectively. The males consumed significantly ($P < 0.05$) higher F' through drinking water than females.

Monsoon :

During monsoon season the mean intake of F' ranged from 2.52 mg/day in normal females and 4.42 mg/day in fluorotic males. No difference was observed in the normal male and females. All other groups showed significant

Table 13 : Seasonal variation in daily fluoride intake from drinking water

Seasons		F' intake (mg/day) Subjects			
		Male		Female	
		Normal	Fluorotic	Normal	Fluorotic
Summer	Range	2.0-11.0	2.5-11.0	1.5-8.8	2.0-8.8
	Mean ± SE	5.35±2.11	6.16±2.16	3.86±1.66	4.87±1.64
Monsoon	Range	1.8-6.0	2.5-6.2	1.4-4.0	2.0-4.9
	Mean ± SE	3.00±1.11	4.42±1.04	2.52±0.77	3.69±0.90
Winter	Range	0.5-1.8	0.4-2.0	0.4-1.2	0.5-1.5
	Mean ± SE	0.84±0.34	0.93±0.36	0.74±0.23	0.76±0.23

CD at 5% level = 0.69

variations. The increasing order of water F' intakes per day was, normal females / normal males / fluorotic females / fluorotic males. In each sex the fluorotic subjects' F' intake values were significantly (P / 0.05) higher than the normal subjects F' intake values.

Winter :

During this season the water F' intake values of non-fluorotic (0.84 mg/day) and fluorotic males (0.93 mg/day). Similarly in females the fluorotics 0.74 mg/day) and the normals (0.76 mg/day). No significant differences were observed between the fluorotic and normal males and females subjects. The seasonal variation trends reveal that in monsoon and summer all the subjects consumed significantly (P / 0.05) higher F' through water as compared to that consumed in winter.

4.14 Seasonal variation in mean daily F' intake through food excluding tea of fluorotic and normal limits :

The data on seasonal effects on daily food F' (excluding tea) intake are given in Table-14.

Summer :

During this season all the four groups of subjects were found to consume similar F' content through food. The maximum mean F' intake of 2.10 mg/day was observed in fluorotic males through this source.

Monsoon :

The fluorotic males (4.30 mg/day) consumed significantly (P/ 0.05) higher amount of F' than the normal females (3.24 mg/day) in this season. The F' intake of fluorotic and normal males and fluorotic females was similar through this source.

Winter :

The maximum (8.53 mg/day) intake was found in fluorotic males

Table 14 : Seasonal variation in mean daily F' intake through food excluding tea of fluorotic and normal rural adults

Seasons		F' Intake (mg/day)			
		Subjects			
		Male		Female	
		Normal	Fluorotic	Normal	Fluorotic
Summer	Range	0.5-3.5	0.5-5.6	0.5-5.2	0.5-3.3
	Mean ± SE	1.50±1.26	2.10±1.27	1.46±1.14	1.51±0.82
Monsoon	Range	2.0-7.5	2.4-7.1	1.8-4.9	2.0-6.0
	Mean ± SE	3.74±1.42	4.30±1.48	3.24±0.85	3.49±1.12
Winter	Range	3.9-12.5	4.4-18.7	3.6-11.8	4.1-13.5
	Mean ± SE	6.60±3.05	8.53±3.34	5.26±1.85	6.89±2.28

CD at 5% level = 1.02

and the minimum (5.26 mg/day) in normal females. In both sexes the fluorotic subjects consumed significantly (P $\angle$ 0.05) higher amount of F' than their normal counterparts. The males consumed significantly (P $\angle$0.05) higher food F' than the females in both the normal and fluorotic category.

The seasons affected the F' intake of all four groups F' significantly. The F' intake values in winter were significantly (P $\angle$ 0.05) higher than those of monsoons which in turn were significantly (P $\angle$ 0.05) higher than those of summer.

4.15 Seasonal variation in daily F' consumption through tea of fluorotic and normal rural adults :

From data in Table-15, it is evident that the mean tea F' intake during different seasons ranged from 0.63 to 4.80 mg/day. The seasonal effects are as given below :

Summer :

In summer season the four groups of adults consumed similar amounts of F' from the tea per day. The highest value of 1.02 mg/day was for the fluorotic males. The fluorotic females and normal males consumed 0.70 and 0.73 mg/day of F' from the tea, respectively. The lowest value of tea consumption F' was 63 mg in normal females.

Monsoon :

The normal fluorotic females consumed similar amount of tea F' per day. However, the fluorotic males consumed significantly (P $\angle$ 0.05) higher tea F' levels than normal males. The tea F' intakes per day of fluorotic males were also significantly (P $\angle$ 0.05) higher than those of all the females however no differences in the tea F' intakes existed between the normal male, normal female and the fluorotic female.

Table 15 : Seasonal variation in daily F' consumption through tea of fluorotic and normal rural adults

Seasons		F' Consumption (mg/day) Subjects			
		Male		Female	
		Normal	Fluorotic	Normal	Fluorotic
Summer	Range	0.3-2.7	0.3-2.7	0.3-2.7	0.3-1.8
	Mean ± SE	0.73±0.71	1.02±0.71	0.63±0.54	0.70±0.56
Monsoon	Range	1.2-4.8	1.5-4.6	1.0-3.3	1.2-3.5
	Mean ± SE	2.24±0.87	2.74±0.79	1.96±0.56	2.04±0.64
Winter	Range	1.6-7.2	2.0-8.0	1.6-5.3	1.8-6.0
	Mean ± SE	3.38±1.30	4.80±1.49	2.76±0.86	4.03±1.00

CD at 5% level = 0.40

Winter :

In winter the fluorotic subjects consumed significantly ($P \angle 0.05$) higher tea F' levels per day than the normal subjects in both sexes. The fluorotic males were found to consume significantly ($P \angle 0.05$) higher tea F' per day not only in comparison to the normal counterparts, but also in comparison to both the categories of female subjects.

Significant effect of seasons was observed on the daily tea F' intake of all the four categories of subjects. The winter values were significantly ($P \angle 0.05$) higher than the monsoon values which in turn were significantly ($P \angle 0.05$) higher than summer values of all the four categories of subjects.

4.16 Fluoride contribution from food, drinking water and tea in daily diets of rural adults :

Data in the Table-16 showed the F' contribution from food, drinking water and tea in daily diets of rural adults.

Summer :

During summer season the maximum mean F' intake was 9.28 mg/day in fluorotic males and minimum (6.95 mg/day) in normal males. It was revealed that F' contribution through food, drinking water and tea was 20%, 70% and 10%, respectively in all the four groups studied. Drinking water contributes more to the daily intake as compared to food source during this season.

Monsoon :

The mean daily intake of F' was higher in fluorotic males (10.19 mg/day) than that by normal males (8.98 mg/day). The contribution of F' through food was 42% through drinking water was 33% and through tea it was 25%. The mean total F' intake of fluorotic males and females was higher as compared to normal males and females, respectively.

Table 16 : Fluoride contribution from food, drinking water and tea in daily diets of rural adults

Subjects		Daily F' intake											
		Food(mg)			Drinking water (mg)			Tea(mg)			Total		
		Summer	Monsoon	Winter	Summer	Monsoon	Winter	Summer	Monsoon	Winter	Summer	Monsoon	Winter
Male	Normal	1.50 (19.78)	3.74 (41.64)	7.6 (64.29)	5.35 (70.58)	3.00 (33.40)	0.84 (7.10)	0.73 (9.63)	2.24 (24.94)	3.38 (28.59)	7.58	8.98	11.82
	Fluorotic	2.10 (22.62)	4.30 (42.19)	8.5 (64.15)	6.16 (66.37)	3.42 (33.56)	0.93 (7.02)	1.02 (10.99)	2.47 (24.23)	3.80 (28.61)	9.28	10.19	13.23
Female	Normal	1.46 (21.00)	3.24 (41.96)	6.2 (63.91)	4.86 (69.92)	2.52 (32.64)	0.74 (7.62)	0.63 (9.06)	1.96 (25.38)	2.76 (28.45)	6.95	7.72	9.7
	Fluorotic	1.51 (21.32)	3.49 (42.45)	6.8 (64.2)	4.87 (68.78)	2.69 (32.72)	0.76 (7.17)	0.70 (9.8)	2.04 (24.81)	3.03 (28.61)	7.08	8.22	10.59

Figures in parentheses indicate percentage.

Winter :

During winter season the mean total intake of F' was higher than that of both the seasons viz., summer and monsoon. Similarly, the mean intake of F' was higher in case of fluorotic males and females than their respective normal counterparts. The intake through food, drinking water and tea was 64%, 77% and 29%, respectively. The mean total F' intake ranged from 9.7 to 13.25 mg/day. Similar results were reported by Sangha et al. (1991) in rural areas of Faridkot district of Punjab where the mean daily F' intake from all the sources including food, water and tea was 19 mg in men and 13 mg in women. The F' concentration in drinking water was found to be more (2.29± 1.50 ppm) than recommended level. Lakdawala and Punekar (1973) said that food items contributes much to the daily F' intake but their study reported the combined contribution of food and water etc. The study further attributes the differences in food F' intake to be different quantities of food and water consumed by the subjects.

4.17 Seasonal variation in daily total F' intake of fluorotic and normal rural adults :

The data on contribution to total F' intake from both food and drinking water are given in Table-17. The daily intake of F' varied from both food and drinking water during all the three seasons are as below :

Summer :

During summer season the mean total F' intake ranged from 6.32 to 8.26 mg/day. The fluorotic males and females consumed a mean of 2.10 and 1.51 mg/day which is higher than the normal subjects mean total intake. The mean total F' intake through drinking water by fluorotic males and females was 616 and 4.87 and 4.87 mg/day, respectively. The mean total intake of

Table 17 : Seasonal variation in daily total F' intake of fluorotic and normal rural adults

Subjects		Source of F' Food(mg)			Drinking water(mg)			Total		
		Seasons								
		Summer	Monsoon	Winter	Summer	Monsoon	Winter	Summer	Monsoon	Winter
Male	Normal	1.50	3.74	7.6	5.35	3.00	0.84	6.85	6.74	8.44
	Fluorotic	2.10	4.30	8.5	6.16	3.42	0.93	8.26	7.72	9.43
Female	Normal	1.46	3.24	6.2	4.86	2.52	0.74	6.32	5.76	6.94
	Fluorotic	1.51	3.49	6.8	4.87	2.69	0.76	6.38	6.18	7.56

F' of subjects was more through drinking water as compared to that from the food. Kramer et al. (1974) reported that out of total intake of 3 to 4.0 mg/day, 2 mg is contributed from the diet and remainder from the drinking water.

Monsoon :

The mean total intake was maximum (7.72 mg/day) in fluorotic males and minimum (5.76 mg/day) in normal females. During monsoon season the subjects of mean F' intake was found to be almost equal through both food and drinking water. The total F' intake was higher in males as compared to females.

Winter :

During winter season the total intake ranged from 6.94 to 9.43 mg/day. The fluorotic males and females consumed higher amount of F' than normal subjects. The intake through food was higher in comparison to that from water. This may be attributed to less consumption of water during winter season. The mean total F' intake through drinking water by fluorotic male and females was 0.93 and 0.76 mg/day which was higher than the normal males and females 0.84 and 0.74 mg/day, respective.

4.18 Seasonal variation in spot urinary F' excretion of fluorotic and normal rural adults :

Data on seasonal variation in urinary F' excretion are presented in Table-18.

The mean excretion of F' in spot urine samples varied from 2.27 to 6.11 ppm among the four groups of subjects studied. The seasonal variations as given below :

Summer :

The mean F' excretion of 5.80 ppm of normal males was significantly ($P \angle 0.05$) higher than 3.82 ppm of normal females. Similarly, the mean F'

Table 18 : Seasonal variation in urinary F' excretion of fluorotic and normal rural adults

Seasons		Urinary F' Excretion (ppm) Subjects			
		Male		Female	
		Normal	Fluorotic	Normal	Fluorotic
Summer	Range	1.5-12.0	0.9-11.0	1.0-10.2	1.0-6.0
	Mean ± SE	5.80±2.64	4.58±2.25	3.82±2.32	2.99±1.33
Monsoon	Range	2.1-14.0	1.8-11.2	1.2-13.0	0.5-9.0
	Mean ± SE	5.23±2.77	3.90±2.51	3.30±2.36	2.27±1.59
Winter	Range	2.0-15.0	2.0-11.9	2.1-12.0	1.8-10.0
	Mean ± SE	6.11±3.97	4.95±2.46	4.56±2.45	4.30±2.38

CD at 5% level = 1.38

excretion of 4.58 ppm of fluorotic males was significantly ($P < 0.05$) higher than 2.82 ppm of fluorotic females. Both male and female fluorotics did not have significant differences in urinary F' excretion in comparison with their normal counterparts.

Monsoon :

The mean spot urinary F' excretion during monsoon season ranged from 2.27 to 5.23 ppm. The F' excretion of 5.23 ppm and 3.30 ppm with respect to normal male and female subjects were found to be significantly ($P < 0.05$) higher than the values of 3.90 and 2.27 ppm of the fluorotic male and female subjects, respectively. Though the normal male and female subjects appeared to excrete higher urinary F' than their fluorotic counterparts. The differences were not significant.

Winter :

The mean excretion of F' in urine varied from 4.30 to 6.11 ppm. The urinary F' excretion of normal male and female subjects was found to be significantly ($P < 0.05$) higher than the fluorotic subjects. It was further revealed that the F' excretion in winter of all the subjects was found to be higher in winter season than summer and monsoon. However, differences were found to be non-significant.

Similar results were reported by Shukla (1982) who observed that the fluorotics may retain more F' as compared to normal subjects. Teotia and Teotia (1982) reported the urinary F' level to be a misleading parameter for the diagnosis fof fluorosis. According to him after prolonged consumption of high F' possibly kidney tubules fail to function normally and the F' excretion gradually diminishes to normal limits.

4.19 Covariance matrix F' intake vs. spot urinary F' excretion of selected rural adults :

Data in the Table-19 showed the F' intake of subjects to be positively ($P < 0.05$) correlated with urinary F' excretion in all the three seasons and in all the studied subjects. The correlation in summer, monsoon and winter in normal males were 0.467, 0.796 and 0.723, respectively. In the fluorotic males the correlations were found to be 0.615, 0.690 and 0.776 during summer, monsoon and winter seasons. The correlation values for normal and fluorotic females were 0.668 and 0.446, respectively in summer, 0.701 and 0.643 in monsoon and 0.460 and 0.519, respectively in winter.

Table 19 : Covariance matrix F' intake vs. urinary excretion of selected rural adults

Seasons	Subjects			
	Male		Female	
	Normal	Fluorotic	Normal	Fluorotic
Summer	0.467*	0.615*	0.688*	0.446*
Monsoon	0.796*	0.690*	0.701*	0.643*
Winter	0.723*	0.776*	0.460*	0.519*

* Significant at 5% level

CHAPTER-V

SUMMARY AND CONCLUSION

The present investigation was conducted with two major objectives. The first was to study the F' contents of raw foods and potable water of a fluorotic village of Haryana. The second aspect of the study included the estimation of total F' intake of the people and their urinary F' excretion. Special emphasis was on the assessment of the seasonal differences in the underground potable water F' levels, peoples floride intake and urinary excretion. For the purpose of this study the village selected was Gangwa, about 6 KM away from Hisar town. A preliminary survey of the potable water sources viz., municipal water, wells and handpumps was made. The water from all these sources being used for drinking and cooking purpose by the people were collected during three seasons and the depth of the sources noted by enquiries from the villagers, Sarpanch etc. On the basis of presence or absence of dental mottling a sample of 50 fluorotic and 50 non-fluorotic adults with equal number of male and female subjects were selected. Adequate representation of people the drinking water from all sources was ensured. Therefore, the selected respondents represented all 8 groups of people using potable water from 8 sources (7 ground water samples, 1 municipal). Except the raw foods all the other observations were made for all the three seasons i.e. summer, monsoon and winter. The summer sampling was done in the month of May, the monsoon and winter seasons wererepresentedby the months of August and November, respectively. List of all the foods consumed from all the food groups by each respondent was prepared.

Samples of the raw cereals, pulses, condiments, spices and vegetables and tea leaves commonly consumed by the population were collected from the families or from the village shop. The residential history was recorded through a structured pretested questionnaire. Fluoride intake was estimated, for this one fifth sample of all the foods (solid and liquid) consumed by each selected respondent was collected for three consecutive days. This included the drinking water and the beverages like tea, butter milk etc. The total intake was calculated for each respondent and the average of three days calculated. Calculation of F' content through food villa method (1979) was used. The total intake through tea and water, by each individual was also separately recorded. The daily F' intake from these two liquids separately were computed. The spot urine samples were collected of all subjects for three days each during summer, winter and monsoon and analysed for F' content.

Potable water, urine, tea, salt, composite diet samples, pulses and legumes, green leafy vegetables, spices and condiments consumed by the rural population were also analysed for F' content.

All the potable water sources analysed in the village contained F' substantially higher than the safe level of 0.5 ppm. The municipal water also contained more F (1.08 ppm) than the recommended levels. The F' content of wells water ranged from 2.03 to 3.07 and that of handpumps water varied from 2.03 to 4.33 ppm. The depth of wells ranged from 18 to 22 feed.

The maximum (50%) of selected subjects were in the age group of 30 to 40 years. About 80% of the subjects consumed water with F' level of 2.0 to 3.5 ppm, 6% consumed water with less and 14% consumed water more than this level of F'. Out of the raw vegetables, amongst roots and tubers; carrots contained maximum (3.2 ppm) F', amongst green leafy vegetables, spinach had 3.8 ppm F', red chillies had 10 ppm F' on dry matter basis. On fresh matter basis the F' content of potato was maximum (0.45 ppm) followed by carrots (0.44 ppm) and onion (0.40 ppm), respectively.

Among all green leafy vegetables, chana leaves (0.36 ppm), spinach (0.26 ppm) had notably high, while other vegetables had F' content ranging from 0.05 to 0.1 ppm. Among all the pulses and legumes maximum F' was in Bengal gram followed by green gram and black gram which had least F' content. Out of all spices and condiments cardamom contained maximum (12.5 ppm) F'. Rock salt had more F' than the other salt samples.

The F' intake of fluorotic subjects was significantly higher than that of normal subjects during three different seasons. The intake of F' through food and tea in two seasons i.e. summer and monsoon was almost similar in all the subjects. In all the three seasons, the proportion of F' from food, drinking water and tea remained similar for the normal and fluorotic subjects.

The urinary F' excretion of fluorotics was less than the normal subjects. No seasonal differences were found in any group. There was positive correlation of F' intake and spot urinary F' excretion in all the four groups studied.

Regarding relationship of nutritional status with the prevalence of fluorosis 68% each of the fluorotic males and females were mildly to severely undernourished. Fifty two per cent and 44% of non-fluorotic males and females, respectively were mild to severely undernourished. Sixty two per cent of the total subjects had moderate degree of dental fluorosis. 26% had mild and 10% had very mild degree of dental fluorosis was observed.

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Appendix-I

Preliminary dental mottling survey

Clinical Picture of Teeth					
Name of family member	Age	Type of teeth appearance	Normal/problematic (Mottling, Cavities, Caries)	Treatment taken	
				Yes What	No Why
		Primary			
		Secondary			

Appendix-II

Fluoride content of food and potable water and its intake in adults of a fluorotic village

Interview Schedule

Sr.No.

Name :

Address :

I. **General Information**

1. Age ________ Yrs.

2. Caste :

Low (Chamar, Bhangi, Doom, Jhimar, Khati, Dhobi, Badi)

Middle (Lohar, Kumhar, Darji, Nai, Banya, Sonar, Ahir, Julaha, Saini,Arora)

High (Brahmin, Jat, Rajput, Dishnoi)

3. Education

Illiterate

Primary

Middle

High School

Teaching (Vocational Education)

Graduate

Post-graduate & above

4. Type of Family :

Nuclear

Joint

5. <u>Size of Family :</u>

Small (upto 5 members)

Medium (upto 10 members)

Large (above 10 members)

II. Residence History

1(a) Family residence history since the last 20 years

Name of place	Duration of stay in years
1.	
2.	
3.	

1(b) Duration of stay in present house/village __________yrs.

2. Water used for drinking and cooking :

Cooking/Drinking water source	Depth	Water level	Location
Well			
Handpump			
Municipality Tap			
Canal			
Any other			

3. Indicate the spices commonly used by your family :

Badi Ilayachi

Haldi

Red chillis

Jeera

Dhania

Long

Chhoti Ilayachi

Kali Mirch

Dal chini

Garlic

Ajwain

Garam Masala

Sonth

4. Tea consumption related information :

Brand of tea being used

a) Unbranded

b) Taj Mahal

c) Brook Bond Red Label

d) Tata Tea

e) Gulner

f) Lipton Richbru

g) Keep changing

5. Which type of salt do you use in cooking ?

Brand of stalt being used

a) Tata Salt

b) Iodized salt

c) Bharat salt

d) Unbranded

h) Unground

6. What type of milling procedure is used for :

	Stone hand grinder	Store electric	Electric grinder
Wheat flour			
Bengal gram flour			
Bajra			
Any other			

7. <u>Commonly consumed green leafy vegetables</u>

Name of leafy vegetables	Months when consumed										
	Jan.-Feb.	March	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Bathua											
Patta Gobhi											
Carrot leaves											
Dhania											
Methi											
Paudina											
Sarson Ka Saag											
Mulee Ka Patta (Radish leaves)											
Palak											
Cowpea pods											

8. <u>Degree of dental fluorosis</u> :

a) Questionable

b) Very mild

c) Mild

d) Moderate

e) Severe

Appendix-III

Day 1/2/3 Food intake - Summer/Winter/Monsoon

Meal	Menu	Total intake (g/ml)	Sample collected (g/ml)
Bed-Tea			
Breakfast			
Lunch			
Evening Tea			
Dinner			

Appendix - IV (A)

Analysis of variance for potable water F' intake levels

Source	d.f.	S.S.	M.S.	F
Between groups	10	74.2934	6.75	250.146**
Within group	70	1.942	0.0277	
Total	80	76.2356		

CD at 5% level = 0.09

Appendix - IV (B)

Analysis of variance for F' intake through food

Source	d.f.	S.S.	M.S.	F
Between group	11	1746.01	158.72	46.8**
Within group	288	974.948	3.3852	
Total	299	2720.956		

CD at 5% level = 1.02

Appendix-IV (C)

Analysis of variance for F' intake through drinking water

Source	d.f.	S.S.	M.S.	F
Between group	11	1050.41	95.49	60.82**
Within group	288	451.996	1.569	
Total	299	1502.408		

CD at 5% level = 0.69

Appendix-IV (D)

Analysis of variance of F' intake for tea

Source	d.f.	S.S.	M.S.	F
Between group	11	329.91	29.99	37.49**
Within group	288	230.653	0.800	
Total	299	560.563		

CD at 5% level = 0.40

Appendix-IV (E)

Analysis of variance for urinary excretion of F'

Source	d.f.	S.S.	M.S.	F
Between group	11	353.68	32.15	5.15**
Within group	288	1799.240	6.247	
Total	299	2152.929		

CD at 5% level = 1.38