

## Uptake of Fluoride, Aluminum and Molybdenum by Some Vegetables from Irrigation Water

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**ABSTRACT** Water is the major source of fluoride (F) in fluorosis endemic areas although food materials also contribute considerable amount to total intake. Plants take up F from irrigating waters and this uptake is influenced by some inorganic constituents in water and soil. In the present study five commonly consumed vegetables [amaranth (*Amaranthus gangeticus*), spinach (*Coriandrum sativum*), cabbage, tomato and lady's finger] were grown applying irrigation water containing 10 ppm F. In addition amaranth and coriander were grown with aluminium (Al) and/or molybdenum (Mo) to study their effect on F bioavailability. Fluoride levels were higher in edible parts of all vegetables compared to controls irrigated with water containing 0.3 ppm F. Fluoride contents (mg/kg dry wt) with tap water and fluoridated water were 0.71 vs 1.70 for spinach, 3.88 vs 20.29 for amaranth, 0.12 vs 0.17 for cabbage, 0.14 vs 0.43 for lady's finger and 0.12 vs 0.2 for tomato ( $P < 0.01$ ). The levels of calcium (Ca) and phosphorous (P) are high in amaranth although their causal relation to high fluoride bioavailability in this plant is not known. Molybdenum (Mo) and Al reduced fluoride intake by amaranth (Al showing marked effect) but not by coriander. Al + Mo reduced fluoride uptake by both plants. Molybdenum intake was reduced by F in coriander and by Al in amaranth. F + Al had much less effect on Mo uptake. Fluoride reduced Al uptake by amaranth and coriander. Mo or Mo + F had no effect on Al uptake by amaranth but reduced the uptake by coriander. Fluoride uptake was highest in amaranth and lowest in cabbage among the vegetables studied.

### INTRODUCTION

Fluorosis is a major public health problem in 18 out of 32 constituent states of India (Susheela, 1999), which is further aggravated by influence of various environmental factors. Incidences of endemic fluorosis are increasing because of altered environmental conditions such as, decrease in rainfall, excessive use of ground water and, lowered ground water level which lead to increase in concentration of different elements including fluoride in ground water. Deposition of fluoride in the bone is influenced by several dietary factors such as protein, calcium, copper, zinc, and molybdenum (Reddy et al., 1972). All of these have negative correlation with deposition of fluoride in the body except molybdenum which has a positive correlation (Quarterman et al., 1979). Molybdenum improves the growth of some crops and use of commercial fertilizers having Mo may extend the problem to areas that were unaffected till now (Blanc, 1976). Endemic genus valium has been

identified as a manifestation of chronic fluoride toxicity in some parts of India (Krishnamachari et al., 1974). When aluminum and F are present in drinking water, they could form fluoroaluminium complexes, such as  $AlF_4^-$  in the stomach that compound to their ionic forms, would be transported more easily in to blood stream and across the blood brain barrier (Bigay et al., 1987)

Epidemiological studies revealed that the prevalence of genus valium in areas with fluorosis is significantly higher in subjects, whose staple food is sorghum (4%) as compared to those whose staple is rice (1%) (Krishnamachari et al., 1976). Sorghum and pearl millet grown and consumed in fluorosis endemic areas contained significantly higher amount of molybdenum than that grown in non-fluorite areas in India (Deosthale et al., 1977).

The major part of fluoride ingested in areas endemic to fluorosis is water, although some food materials contribute considerable amount to total intake (Walbott, 1963; Singh and Ophague, 1979; Nawlakhe, 1981; Gulati et al., 1993; Singh et al., 1993). Crops grown in close proximity to certain factories, especially those producing phosphate fertilizer, steel, aluminum, magnesium, glass and bricks contain more fluoride since fluoride and heavy metal containing dust emitted into air settle on plants (Oelschlager, 1974).

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Fluoride is more soluble in acid soils due to which its uptake by plants is enhanced (Daines et al., 1952).

Liming the soil with calcium oxide reduces F accumulation (Trace, 1954). Food materials collected from fluorotic areas were found to contain higher levels of molybdenum (Deosthale and Gopalan, 1974) it means F promotes accumulation of Mo. Plants grown in clay loam take up less fluoride than those grown in sand. Among items of plant origin, tea leaves contain significant amounts (30 - 300 mg/g dry weight) of fluoride. Bioavailability of F to plants could be influenced by several metal ions due to complexation, precipitation, pH changes etc. Aluminum and Mo were selected in this study for reasons that follow. Earlier reports show that the accumulation of fluoride by tea plants is facilitated by formation of complexes of aluminum with fluoride (Devison, 1984). Molybdenum is known to increase copper excretion (Buck et al., 1973) and copper deficiency could in turn lead to derangement of bone metabolism (Suttle et al., 1972). Although aluminum is the most abundant metal in nature, it has no known biological function. However, it is known that there is a causal role for aluminum in dialysis encephalopathy, microcytic anemia, and osteomalacia. Aluminum has also been proposed to play a role in the pathogenesis of Alzheimer's disease (AD) in human even though this issue is controversial. The exact mechanism of aluminum toxicity is not known but accumulating evidence suggests that the metal can potentiate oxidative and inflammatory events, eventually leading to tissue damage in human. However, the effect of fluoride on molybdenum and aluminum uptake by plants is not known. Amaranth and coriander were selected for the study since amaranth contains more aluminum and coriander contains more molybdenum compared to other commonly consumed leafy vegetables by human being. The mechanism of uptake of fluoride by plants and the interaction of fluoride, aluminum and molybdenum on each other's uptake by plants require detailed study.

In this study fluoride absorption from irrigation water by commonly used vegetables (by human being) like amaranth, spinach, tomato, cabbage and lady's finger is studied in *experiment a*. In *experiment b* the effect of molybdenum (Mo), aluminum (Al) and fluoride

on each others absorption by amaranth (*Amaranthus gangeticus*) and coriander (*Coriandrum sativum*) has been investigated.

## MATERIALS AND METHODS

**Experiment a:** Studies on F uptake and accumulation were conducted using green leafy vegetables (spinach, amaranth, cabbage) and other vegetables (tomato and lady's finger). Fluoride (as NaF) at a level of 10 ppm in irrigation water was used for the study. Red soil and cow dung compost (3: 1) was mixed thoroughly, and debris such as sticks and small pieces of brick and stone were removed. Two types of clay pots were used in the study, one type was small (25 cm diameter, 24.5 cm height, soil dung mixture 10.5 kg) and another type was big (32 cm diameter, 29.5 cm height, solid dung mixture 22.5 kg). Small pots were used to grow spinach, coriander and amaranth while big pots were used to grow lady's finger, cabbage and tomato. The drainage was provided through a hole at the bottom of the pot and a cotton plug was used to prevent out flow of soil. Seeds of amaranth, coriander, spinach (15 each per pot), cabbage (2 per pot), tomato and lady's finger (3 each per pot) were sown in labeled pots. All the plants received plain tap water from the metropolitan water supply (F=0.3 pip) as irrigation water for the first 15 days after sowing. Tap water from the metropolitan water supply as such (control, fluoride content 0,3 pip) and with added F (10 pip) was used to water the plants (250 ml/pot/day) in different pots for the period indicated in Table 1.

**Experiment b:** Thirty two small pots were used to grow amaranth and coriander (15 seeds/pot) of each treatment. Municipal tap water (250 ml) having different solutes (F, Al and Mo) was used to water the plants in different pots for the period indicated (Table 2).

After the specified period, plants from *experiment a and b* were harvested, washed thoroughly in tap water, followed by three washes with double distilled water and weights of edible portions were recorded. For calculation of the moisture content, the samples were dried to constant weight in a hot-air oven at 60°C. The dry samples were powdered in a mortar, and stored for F, Al and Mo analysis subsequently. Plants of treatment group were pooled together for analysis and triplicate analyses were done

for obtaining mean ± SD values. Fluoride in dried powders of all vegetable samples in *experiment a* and *b* were estimated by the method of Villa, 1979 using an Orion ion selective electrode (Orion EA 940, Boston MA), Al and Mo in *experiment b* were estimated by direct current plasma emission spectrophotometer (Becmman spectra span V)

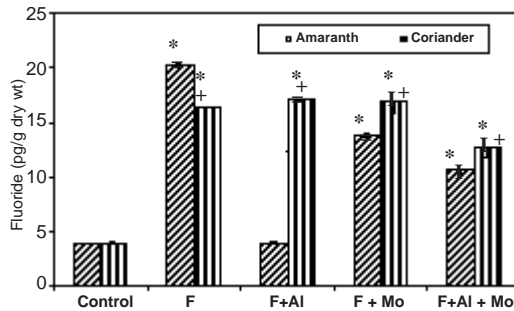
**Statistical Analysis:** Comparison between groups and vegetables were done by ANOVA and Post Hoc Test. The levels of significance were determined, at P= 0.05 and P=0.01 were considered as significant.

**RESULTS**

**Experiment a:** Among the vegetables studied the percent moisture content was found to be highest in Tomato and lowest in amaranth (Table 1). The moisture content among the vegetables did not differ with the F content of the irrigation water. Accumulation of fluoride (Mean ± SD) in the edible portion of the vegetables is shown in Table 1. With tap water containing 0.3 pip F (control), the F content was highest in amaranth and the lowest in cabbage. The fluoride content of all vegetables grown with tap water was comparable to earlier reported values. Among green leafy vegetables, amaranth accumulated a significantly larger (P<0.01) amount of F at 10 pip F level. Cabbage accumulated significantly less (P<0.01) F at 10-ppm level. Fluoride uptake by tomato and lady’s finger was also low at 10 pip F exposure. Spinach, which is consumed in large quantities, took up less F than either amaranth or coriander

**Experiment b:** The normal fluoride content of coriander and amaranth grown on tap water was 3.97 ± 0.02 mg/g dry weight and 3.9 ± 0.02 mg/g dry weight respectively (Fig. 1). When fluoride containing water was given, fluoride content was increased significantly (P<0.01) in coriander and amaranth as compared to controls. The comparisons were done with all the treatments in respect to F treatment alone. Aluminum did not affect fluoride uptake by coriander but significantly reduced (p<0.01) the uptake by amaranth. Molybdenum also did not affect fluoride uptake by coriander but significantly inhibited (P <0.01) the uptake by amaranth. Aluminum and molybdenum, when added together, reduced fluoride uptake by coriander and amaranth significantly (Fig. 1).

The normal aluminum content of coriander and amaranth given tap water was 800 ± 20 mg/



**Fig. 1. Effect of aluminum and molybdenum on fluoride uptake by Amaranth and Coriander Plants**

\*significantly higher (p<0.001) as compare to control.  
+significantly different (p<0.01) as compare to amaranth.

**Table 1: Accumulation of fluoride in edible portion of various vegetables irrigated with 10 ppm fluoride containing water.**

	Spinach		Amaranth		Cabbage		Okra		Tomato	
	Control	Experi-mented	Control	Experi-mented	Control	Experi-mented	Control	Experi-mented	Control	Experi-mented
Total edible mass wt. (gm)	199	190	26.63	27.60	200	200	34.6	33.2	160.9	159
Moisture content %	93.6	93.5	83.89	83.56	93.0	93.0	89.7	89.7	93.9	94.0
Fluoride exposure total days	42	42	40	40	167	167	49	49	54	54
Total fluoride exposure (mg)	-	105	-	100	-	417	-	100	-	135
Fluoride accumulated (mg/kg drywt)	0.71	1.700	3.88	20.29	0.12	0.17	0.14	0.43	0.12	0.2
Percent fluoride accumulation	±0.003	±0.005	±0.1	±0.01	±0.009	±0.01	±0.007	±0.02	±0.005	±0.001
	-	1.61	-	20.30	-	0.04	-	0.43	-	0.14

g and  $900 \pm 30$  mg/gm dry weight respectively (Fig.2). In the plants given aluminum containing water, aluminum increased in coriander as well as in amaranth. The comparisons were done with all the treatments in respect to Al treatment alone. Fluoride affected aluminum uptake by coriander significantly ( $p < 0.01$ ) but not in amaranth. Molybdenum had significant effect on aluminum uptake by coriander ( $p < 0.01$ ) but not by amaranth. Fluoride and molybdenum when added together, reduced aluminum uptake by coriander significantly ( $p < 0.01$ ) but not by amaranth (Fig. 2).

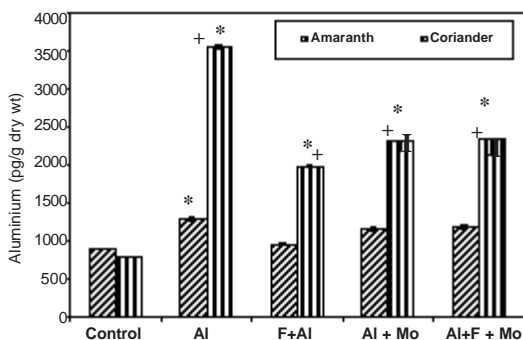


Fig. 2. Effect of fluoride and molybdenum on aluminium uptake by Amaranth and Coriander Plants

\* significantly higher ( $p < 0.001$ ) as compare to control

+ significantly higher ( $p > 0.01$ ) as compared to coriander than amaranth

The normal molybdenum content of coriander and amaranth given tap water was  $9.08 \pm 0.02$  mg/g and  $1.47 \pm 0.02$  mg/g respectively (Fig.3). In the plants given molybdenum containing water, molybdenum increased in

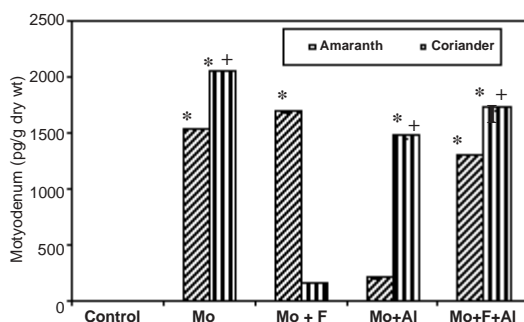


Fig. 3. Effect of fluoride and aluminium on molybdenum uptake by Amaranth and Coriander Plants

\*significantly higher ( $p < 0.001$ ) as compare to control.

+significantly different ( $p < 0.01$ ) as compare to amaranth.

coriander as well as in amaranth as compared to controls. The comparisons were done with all the treatments in respect to Mo treatment alone. Fluoride reduced molybdenum uptake by coriander significantly ( $P < 0.001$ ), while in amaranth fluoride increased molybdenum uptake. Aluminum reduced molybdenum uptake by amaranth significantly ( $P < 0.01$ ) but had no effect on coriander. Fluoride and aluminum when added together, reduced molybdenum uptake by amaranth but increased in coriander.

## DISCUSSION

The F content of food materials reported in the literature shows considerable variations attributable to methodologies and composition of water and soil on which they are grown (Machle et al., 1939; In general, the F content of vegetables, cereals and pulses is not high in comparison to some others like tea leaves Waldboff, 1963). However, the results of the present study show that the F level of some vegetables can be markedly influenced by the F content in irrigation water. All vegetables do not accumulate F to the same extent and variations among vegetables are significantly high. The differences between spinach, amaranth and between cabbage and lady's finger are noteworthy.

Epidemiological studies indicate that the incidence and severity of hydrofluorosis does not show a consistent correlation with the F level in drinking water. In some regions, skeletal and dental fluorosis is rampant with drinking water F level not exceeding 2 to 3 pip. In some other areas where the water F level is as high as 8 pip, the incidence of fluorosis is rare (Krishnamachari, 1976). Although the availability of F from drinking water is almost complete ( $> 90\%$ ), there can be significant variations in the F availability from food materials depending on their composition and soil conditions in which they are grown. Fluorine exists predominantly as fluoride in water supplies. We do not have adequate information on the nature of F in food materials. The possibility of the presence of organic F or tightly bound F in food materials remain to be verified. The existence of fluorosilicates, fluorophosphates, fluorosulphonates, fluoromolybdates, etc. is known, but the occurrence in plant kingdom remains to be worked out.

The results of the *experiment a* of the present study indicate that the F intake from plants foods grown with F rich waters in endemic areas can be a contributory factor to the onset and progression of fluorosis. With foods like amaranth, F intake could add up significantly to that derived from water. The calcium content of vegetables may influence F accumulation because of the affinity of calcium ions for F but such possibility requires further evaluation. In this study amaranth with calcium content of 397-mg/100g (Table 2) showed higher accumulation of F followed by coriander, which contains 184 mg/100 g. However, vegetables with lower calcium content do not show such a correlation. Tomato accumulated much less F, although leafy and root vegetables are reported to accumulate comparatively more F (Singh et al., 1995). Thus from the *experiment a* it can be concluded that the consumption of tomato and lady's finger is unlikely to contribute appreciably to F load even if they are grown in an endemic area. Further work is needed to understand these interrelations.

**Table 2: Fluoride uptake by vegetables compared to their normal levels of calcium and phosphorus**

Name of vegetable (mg/100 g)	Calcium (mg/100 g)	Phosphorus (mg/kg dry wt)	Fluoride content	
			Control	10 ppm
Amaranth	397	83	3.88	20.29
Cabbage	39	44	0.12	0.17
Lead's finger	35	66	0.14	0.43
Spinach	26	73	0.71	1.70
Tomato	20	36	0.12	0.20

Results of the *experiment b* show that both coriander and amaranth accumulate fluoride from water. Metal ions like aluminium and molybdenum affect fluoride absorption and vice versa. Fluoride absorption is reduced by aluminium in amaranth but not in coriander. The behaviour of amaranth is not in conformity with the proposed mechanism of absorption of fluoride by tea leaves. Our preliminary study (unpublished) showed that fresh tea leaves did not contain high fluoride but processed tea from the same garden contained high fluoride which could possibly be derived from materials used during processing. Fluoride increased molybdenum uptake in amaranth as reported earlier but did not do so in coriander. Quantitatively, consumption of coriander as a flavouring agent and spice

is much less compared to amaranth which is consumed in bulk as green leafy vegetable. It is known that molybdenum increases urinary copper excretion (Krishnamachari, 1976; Miller et al., 1999) leading to adverse effects on bone metabolism ( Michle et al., 1939). Aluminium reduced molybdenum uptake by amaranth significantly but not by coriander. Fluoride and molybdenum reduced aluminium uptake by amaranth but not appreciably by coriander. Aluminium decreased fluoride uptake by amaranth, but it may not be advisable and practical to use aluminium salts in the field to reduce fluoride uptake, as accumulation of aluminium in plants may exceed permissible limits.

From *experiment a* and *b* it can be concluded that the F absorption from irrigation water was higher in amaranth and lower in cabbage. Aluminium and Mo reduced fluoride intake by amaranth while in coriander no such effect was observed.

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