EFFECTS OF FLUORIDE POLLUTION ON *MEDICAGO SATIVA* L.

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ABSTRACT: The effects of fluoride air pollution, the most phytotoxic of the common air pollutants, on *Medicago sativa* L. (alfalfa, lucerne) were evaluated. Samples of *Medicago sativa* L. polluted by fluorides emitted from an aluminum production plant in Iran and control non-polluted plant samples were collected and analysed for fluoride ion (F) content using an F ion-selective electrode. Seventy-four qualitative and quantitative leaf characteristics (71 morphological, 2 anatomical, and 1 fluoride content) were studied with the data being encoded and analyzed using the Chi-squared method. The results showed the polluted plants had an increased F concentration and morphological changes, including decreased organ sizes, compared with the controls. The accumulation of F in leaf tissues and the leaf morpho-anatomical changes were considered to be adaptive reactions to F pollution which made *M. sativa* more tolerant to fluoride pollution.

Keywords: Aluminum reduction; Arak, Iran; Fluoride air pollution; *Medicago sativa* L.

INTRODUCTION

The fluoride ion (F) is an injurious pollutant which may adversely affect plants, animals, and humans. Significant sources of F include glassworks, aluminum plants, fertilizer plants, steel mills, brickworks, ceramics plants, coal combustion, and also groundwater, depending on the topsoil type.[1-7] F can be accumulate in the organs of plants[8] and the level of F accumulation in plant leaves can be used to monitor atmospheric HF concentrations.[9,10]

F contamination leads to various morphological changes in plants. Contaminated abnormal lichens contained higher F concentrations compared to normal lichens.[11] Also, F decreases pollen viability and changes pollen morphology in some legumes.[12] Environmental F pollution results in several changes in leaf features such as stomata (size and density), trichomes (length, type, and density) and the subsidiary cells.[13] The polluted leaf tissues had more and longer trichomes compared to the control samples.[14-17] Franzaring et al. observed a negative correlation between plant biomass and the F concentration.[2] Thus, these characteristics can be used as indicators of environmental F pollution.[2]

Weinstein and Davison cited *Medicago sativa* L. (alfalfa, lucerne) as a plant which was tolerant to atmospheric F pollution. In the present study, the effects of F on the morpho-anatomical characteristics of *M.sativa* were investigated.[10]

MATERIALS AND METHODS

The IRALCO (Iran Aluminum Company) is situated in the northeastern region of Arak, Iran (longitude 34º06'N, latitude 49º46'E) on an area of 232 hectares. The annual temperature varies between +39.4ºC and –23.6ºC.[18]

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Mature fresh leaves of *M. sativa* were collected from the IRALCO area from plants growing adjacent to the pot rooms. Control samples of mature fresh *M. sativa* leaves were collected on the same day at a distance of 10 km from the factory. A potentiometric method by the Association of Official Analytical Chemists[19] was followed for preparing the plant samples for the F determinations using an ion-selective electrode (P/N: FQQl502-QQ3B) based on Jacobson[20] and Campbell.[21] The leaf macro- and micro-morphological and the para-dermal anatomical studies were done using fresh, dried, and stored samples. Seventy-four qualitative and quantitative leaf characteristics (71 morphological, 2 anatomical, and 1 fluoride content) were studied in the leaves of 20 plants from the polluted area and compared to control leaves using a zoom binocular light microscope (Leica Galen III), stereo microscope (Blue Light Industry), and scanning electron microscope (SEM JEOL JSM-6100)(Table 1 and Figure 1). The encoding data were analyzed using the Chi-square test (SPSS).

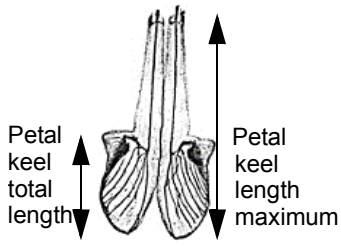

Figure 1. Measurement details for the petal keel total length (character number 42 in Table 1) and the petal keel length maximum (character number 44 in Table 1).

RESULTS AND DISCUSSION

The polluted plants from the IRALCO area had a high F concentration (226±1.07 ppm) compared to the control plants (24±1.32 ppm), while the normal F values for legumes are probably 5 to 10 ppm.[22] This data showed that the polluted *M. sativa* accumulated 9.42 times more F than the control plants. Miller et al. reported 130 ppm F in *M. sativa* L. in a contaminated area.[23] There are some literature reports of F accumulation in plants near F-emitting factories such as an aluminum plant.[2,24-26] Plants take F from the air via the leaves and from the soil via the roots, but sensitivity analysis showed that the accumulation of F is dominated by the atmospheric loading.[24] The maximum and the minimum values for the F content of the air in Arak city, around the aluminum production plant, were 390.1 and 3.2 $\mu g/m^3$, respectively.[26] Variations in F uptake and deposition in plant foliage occur in different plant species, probably due to differences in the leaf surface properties.[27]

The para-dermal anatomical studies showed that the average length and the density of the trichomes increased in the contaminated leaves (Table 2 and Figure 2). It appeared that the changes in the trichomes were an adaptation to the F pollution for reducing the effect of the F on the leaves. The longer trichomes might act as filters and insulators to keep the dust particles away from the ostioles. These features can be used as indicators of the degree of environmental F pollution in these areas.[12] Increased leaf trichome length and density have also been found in other studies[28] together with differentiation in the epidermal characteristics between the polluted and non-polluted samples.[16] Stomata density decreased on the leaf abaxial surface of the polluted *M. sativa* but increased on the adaxial surface (Table 2 and Figure 2).

Table 1. The quantitative and qualitative characters used for the biometry in the control and polluted *M. sativa* samples

1	Plant total length maximum (mm)	38	Wing width (mm)
2	Number of main branches	39	Wing width min (mm)
3	Number of leaves in original branch	40	Wing length (mm)
4	Internodes number in original branch	41	Wing claw length (mm)
5	First internodes length (mm)	42	Keel total length (mm)
6	Diameter of stem in first inter node (mm)	43	Keel limb length (mm)
7	Thickness of stem in last internodes (mm)	44	Keel length max (mm)
8	Secondary internodes length (mm)	45	Keel claw length (mm)
9	Pedicel length (mm)	46	Lable total length/lable limbe width maximum
10	Diameter of pedicel (mm)	47	Calyx length (mm)
11	Diameter of peduncle (mm)	48	Calyx width (mm)
12	Leaf length maximum (mm)	49	Calyx teeth length (mm)
13	Leaflet length maximum (mm)	50	Calyx length max/calyx width maximum
14	Leaflet width maximum (mm)	51	Pistil total length (mm)
15	Lateral leaflet length maximum (mm)	52	Ovary length (mm)
16	Leaflet width maximum (mm)	53	Style length (mm)
17	Leaflet width minimum (mm)	54	Anther length (mm)
18	Petiole length (mm)	55	Filament length (mm)
19	Petiole diameter (mm)	56	Filament width max (µm)
20	Petiolule length (mm)	57	Filament connection length maximum (mm)
21	Thickness of petiolule (mm)	58	Filament connection length minimum (mm)
22	Leaf length/petiole length	59	Ovary/style length
23	Stipule length (mm)	60	Legume length maximum (mm)
24	Stipule width (mm)	61	Legume width maximum (mm)
25	Trichome density on adaxial surface of leaflet /mm^2	62	Legume length/width
26	Trichome density on abaxial surface of leaflet /mm^2	63	Diameter of legume max (mm)
27	Trichome length (µm)	64	Seed number in legume
28	Stomata density on adaxial surface of leaflet /mm^2	65	Seed length (mm)
29	Stomata density on abaxial surface of leaflet /mm^2	66	Seed width (mm)
30	Lable total length (mm)	67	Seed diameter (mm)
31	Lable limbe width (mm)	68	Hilum length (µm)
32	Lable limbe width min (mm)	69	Hilum width (µm)
33	Lable limbe length (mm)	70	Chlorosis: present=1, absence=2
34	Lable limbe claw length max (mm)	71	Necrosis: present=1, absence=2
35	Inflorescence length (mm)	72	Epiderms cells: smooth and regular=1, not smooth and irregular=2
36	Number of flower in inflorescence	73	Stomate condition: normal stomate=2, deep stomate=1
37	Wing total length (mm)	74	Fluoride content (ppm)

Table 2. Comparison of the epidermal leaf anatomical characteristics of the trichomes and stomata in the control and polluted leaves of *M. sativa* using a bright field microscope (Values are mean±SD)

Para-dermal characters	Control leaves	Polluted leaves
Trichome length (µm)	447.50±3.54	450.00±28.28
Trichome density in adaxial surface of leaflet/mm^2	0.00±0.00	0.00±0.00
Trichome density in abaxial surface of leaflet/mm^2	15.38±0.71	21.15±1.77
Stomata density in adaxial surface of leaflet/mm^2	269.23±3.54	309.61±8.13
Stomata density in abaxial surface of leaflet/mm^2	209.61±1.06	176.92±1.41

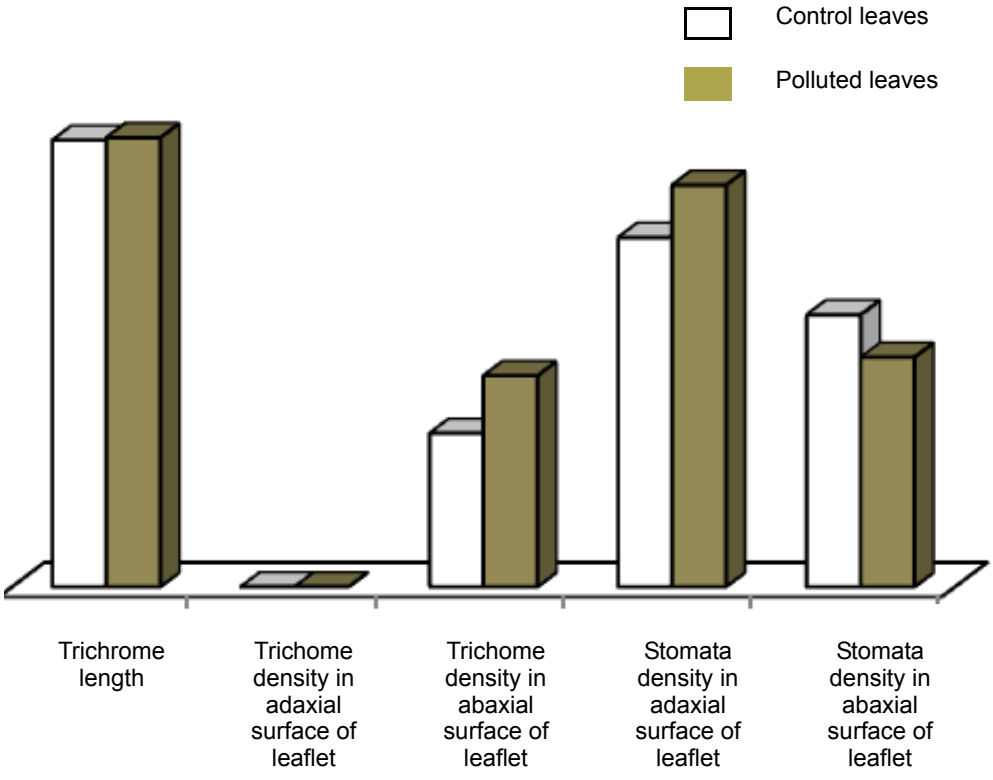

Figure 2. A comparison of the trichome and stomata characteristics in polluted and non-polluted *M. sativa* leaves,

Our scanning electron microscope studies showed there are deep stomata in contaminated samples (Figures 3A and 3B). Sharma and Butler found folding of the subsidiary cells in the leaf surface of some flowering plants with air pollution.[14]

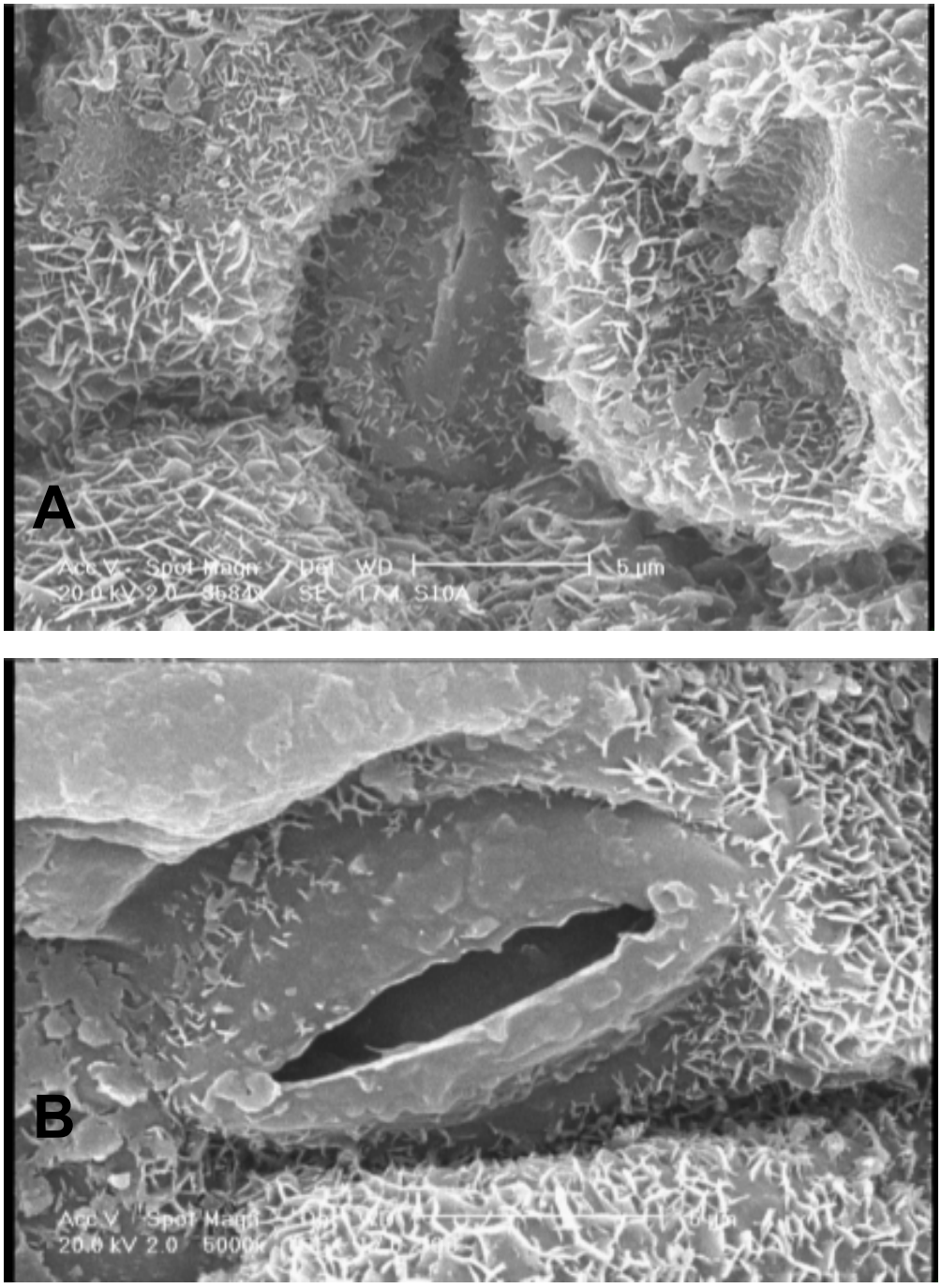

Figure 3A and 3B. Stomata in polluted and non-polluted *M. sativa* leaves. A: deep stomate in contaminated samples 8584×, SEM; B: normal stomate in control sample 5000×, SEM

In the present study, in the polluted *M. sativa* leaves, chlorosis and necrotic spots were found in the leaf tissue at the edges of the leaves and near the veins. In addition, injured and collapsed epidermis was observed in the polluted leaves. The transpiration steam of vascular plants causes huge concentration gradients in the leaves, so that the few millimeters near the tip or the margins may have several hundred times more F than the rest of the leaf.[9]

In the present study, the polluted leaf samples showed a reduction in all of the aerial organ sizes. The plant total length, first internode length, and petal size were all significantly decreased ($p<0.05$) in comparison to the controls. In addition, the seeds were wrinkled, perforated, and without a kernel. Also found in the present study, in the polluted plants, were reductions in the seed size and the number of flowers, leaves, shoots, and internodes in the original branch. (Table 3 and Figure 4).

Table 3. Comprision of the morphological and anatomical characteristics ($p<0.05$) in control and polluted *M. sativa* samples (Values are mean±SD)

Morphological and anatomical characteristics	Control leaves	Polluted leaves
Plant total length	700.00±3.36	624.00±65.0*
First internode length	23.00±0.00	16.00±5.66*
Stipul width	3.00±0.00	2.50±0.00*
Stamen length	7.65±0.21	7.00±0.00*
Petal wing total length	7.55±0.64	7.20±0.00*
Petal wing width maximum	2.55±0.07	2.00±0.00*
Petal keel length	1.45±0.07	1.50±0.00*
Petal keel length maximum	3.70±0.00	3.40±0.00*
Seed width	1.52±0.14	1.30±0.00*

Reports in the literature show that pollution may cause a decline in a number of parameters such as the quantity of flowers and fruits per plant, the number of seeds per fruit, the leaf number and leaf area, the branch numbers, the internode length, the number of nodes and internodes, the basal area of the stem, and the total number of branches.[29-33]

This study showed that *M. sativa* is an accumulator of F and can, in response to F pollution, raise the tissue F concentration. It is believed that various micro- and macro-morphological characteristics can change to help the plant to tolerate F pollution. Sometimes, in spite of high F level in plants, no foliar damage was seen.[34] In *M. sativa* L., an important forage crop in the world, the absorption of fluorides from the air and soil causes significant metabolic changes and visible injuries are induced when the foliar F contents exceeds a specific level. Herbivores feeding on plants with high F levels accumulate F in their tissues.[35,36]

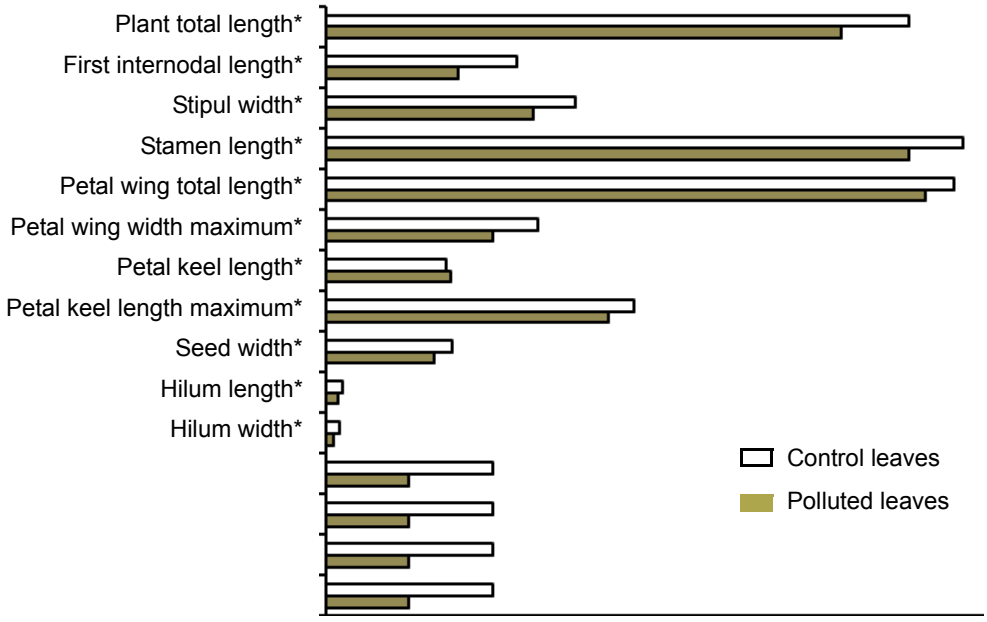

Figure 4. A comparison of the morphological and anatomical characteristics in polluted and non-polluted *M. sativa* samples. Compared to the control leaves, using the Chi-square test (SPSS): *p<0.05.

In turn, the consumption of the meat of livestock which have fed on F-polluted vegetation, by humans or other carnivores, would result in a raised F intake. The excessive intake of F may lead to various disorders including dental and skeletal fluorosis, thyroid impairment, and development neurotoxicity.[37] Therefore, as *M. sativa* is a F accumulator, it should not be used for the grazing of domesticated animals in polluted areas. These contaminated plants ought to be omitted from the food chain. When the source of excess F is irrigation water, defluoridation of the water by various methods is suggested.[4,38]

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