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EFFECT OF FLUORIDE ON THE PHYSIOLOGY AND GROWTH INDICATORS OF MAIZE (*ZEA MAYS* L.)

Sana Ghaffar,[a] Imran Khan,[a,*] Mian Afaq Ahmad,[b] Tafavouq Umar,[a] Iqbal Munir,[b]
M. Nauman Ahmad,[c] Syed Jehangir Shah,[b] Ikram Ullah,[d] Ghulam Mustafa,[d]
Waqar Ali,[e] Toqeer Ahmad[a]
Dera Ismail Khan, Chakdara, Peshawar, and Islamabad, Pakistan

ABSTRACT: The interaction between plants and fluoride occurs due to the contamination by fluoride of air, soil, and ground water. Fluoride pollution is worldwide and may cause serious damage to plants and consequently affect both livestock and human beings. Air pollution by fluoride is widespread in Pakistan and affects crop production. The aim of the present study was to examine the impact in maize (*Zea mays* L.) of 15 days treatment with sodium fluoride (NaF), in concentrations of 0 (control), 10, 20, and 50 mg/L, on germination percentage, root and shoot length, vigor index, total chlorophyll content, and total protein content. The group size was 50 seeds for each analysis. Increasing concentrations of NaF resulted in reductions in the germination percentage, root and shoot length, vigor index, and total protein content. Our finding that the total chlorophyll content increased with a peak at 20 mg/L NaF and a value at 50 mg/L still above the control value was anomalous compared to the literature. It was concluded that fluoride had a negative effect on the crop productivity of *Zea mays* L. and that soil contamination by fluoride should be prevented.

Keywords: Chlorophyll; Fluoride; Growth indicators; Protein; Seed germination; Vigor index.

INTRODUCTION

Fluoride pollution may not only cause severe health problems in animals and humans but also present a serious threat to crop production.[1-6] In plants, the presence of high levels of fluoride in soil and ground water may be the foundation of diseases which may reduce total crop production.[1] Fluorine is a unique chemical element which occurs naturally but is not an essential nutrient for plants and fluoride toxicity can arise from excessive fluoride intake from a variety of natural and man-made sources, including brick production.[2] In plants, fluoride ingestion may have a serious toxic impact on the leaves, reduce germination and growth, and adversely affect various biochemical indicators and metabolic processes including protein metabolism, carbohydrate metabolism, and photosynthesis.[2,3] The presence of fluoride in ground water may directly impact crop productively by producing harmful effects on yield maturity and ripeness, mainly at the stage of seed augmentation.[4,5] A high fluoride content in plants may increase reactive oxygen species (ROS), reduce antioxidant defenses, and damage DNA and proteins.[6] Fluorides may be absorbed by plant roots and then transferred to plant leaves where they produce toxic effects.[7-9] Fluoride contamination of water, mud, and plants is a worldwide problem. In plants, the main effects of fluoride poisoning are chlorosis, burning of leaves. and leaf necrosis.[1]. Fluoride is described as being phytotoxic for plants because it may create toxic outcomes in several metabolic pathways. The sources of soil contamination by fluoride include the use of phosphate fertilizers, environmental contamination from

[a]Gomal Center of Biotechnology and Biochemistry, Gomal University, Dera Ismail Khan, Pakistan; [b]Institute for Biotechnology and Genetic Engineering, Agriculture University Peshawar, Peshawar, Pakistan; [c]Department of Chemistry, Agriculture University Peshawar, Peshawar, Pakistan; [d]SA-CIRBS, International Islamic University, Islamabad, Pakistan; [e]Department of Biotechnology, University of Malakand, Chakdara, Lower Dir District of Khyber Pakhtunkhwa, Pakistan. For correspondence: Dr. Imran Khan, Gomal Center of Biotechnology and Biochemistry, Gomal University, NH 55, Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan; E-mail: drimran@gu.edu.pk

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manufacturing, such as brick kiln use, and from the burning of fluoride-containing fuels.[13]Vegetation may be damaged by exposure to fluoride from phosphate fertilizer manufacturing industries.[14]

The seeds of maize (*Zea mays* L.) are an important source of nutrition worldwide. Maize is the third most grown crop in the world and in Pakistan it is the fourth largest grown crop after wheat, cotton, and rice with the area under maize being over one million hectares and the production approximately 3.5 million metric tons.[15] In Pakistan, maize is mainly grown in Khyber Pakhtunkhwa (KPK) and Punjab.[16] Maize is a rich source of numerous necessary chemical elements also helps to prevent various chronic diseases.[17] The seeds of maize are a source of several vitamins including those in the B, E, and K families.[187]

The aim of the present study was to examine the impact on maize (*Zea mays*) of 15 days treatment with sodium fluoride (NaF), in concentrations of 0 (control), 10, 20, and 50 mg/L, on the germination percentage, root and shoot length, vigor index, total chlorophyll content, and total protein content.

MATERIALS AND METHODS

Sodium fluoride (NaF) was used as a fluoride source during the current research. Healthy maize kernels, sterilized with a 5% solution of sodium hypochlorite (NaOCL), were transferred to Petri dishes containing filter papers soaked with different concentrations of NaF solution, i.e., 0, 10, 20, and 50 mg/L. The sample size for each analysis was 50 seeds. After 15 days, in a plant development compartment, the seedlings were investigated for seed germination percentage, shoot and root measurement lengthwise, and a vigor index measurement. All the seeds were examined in the analysis of the biological and chemical indicators.

Seed germination percentage: The percentage of seed germination was determined using equation 1:

$$\text{Germination percentage (\%)} = \frac{\text{Number of normally germinated seeds}}{\text{Total number of seeds}} \times 100 \quad \text{.........Equation 1}$$

The percentage of vigor indicator (vigor index): The vigor index is a measure of the performance of a sample of seeds throughout the germination event. It was determined for each sample by multiplying the seedling growth with the percentage of seed germination as shown in Equation 2:.

$$\text{Vigor index} = (\text{Root length [cm]} + \text{Shoot length [cm]}) \times \text{Germination percentage (\%)} \quad \text{.. Equation 2}$$

Average root/shoot measurement lengthwise: The root and shoot lengths of the seedlings after 15 days of growth were measured with the help of a meter scale. All measurements, taken in centimeters, were compared to the control seedlings and the effect of fluoride on the root and shoot measurement lengthwise of the plants determined.

Flow sheet: The stages of seed germination and seedling growth of *Zea mays* after treatment with NaF at concentrations of 0,10, 20, and 50 mg/L are shown in Figures 1A–1C.

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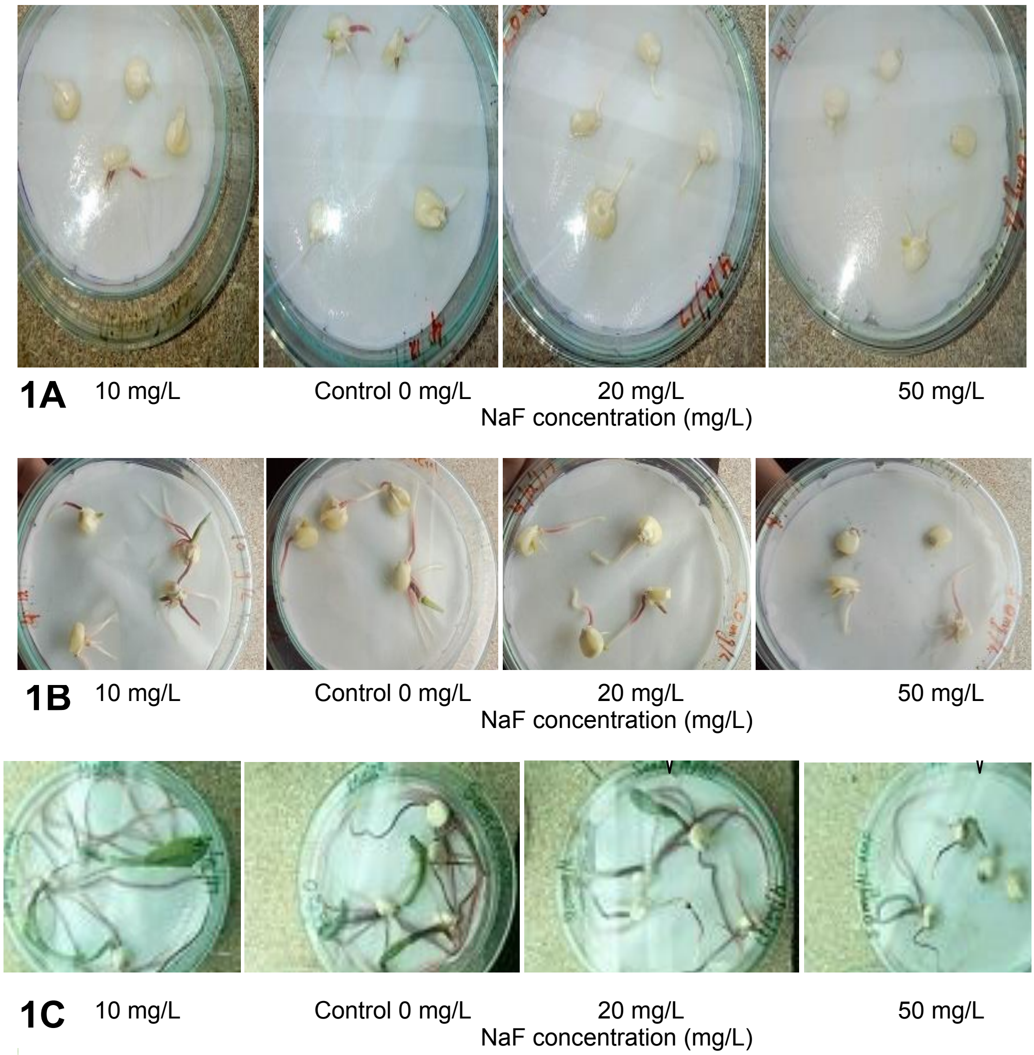

Figures 1A–1C: The stages of seed germination and seedling growth of *Zea mays L.* after treatment, for 1, 2, and 15 days, with NaF at concentrations of 0,10, 20, and 50 mg/L 1A: Germination of seedlings after 1 day of treatment with NaF; 1B: Germination of seedlings after 2 days of treatment with NaF; 1C: Germination of seedlings after 15 days of treatment with NaF.

Determination of total chlorophyll content: For determining the total chlorophyll contents of the fluoride treated plants, 0.5 g of leaf tissues from each sample were weighed and homogenized in 80% acetone. The homogenized contents were centrifuged at 1000 rpm for 15 minutes at a temperature of 4ºC. The resulting supernatants containing the chlorophyll were transferred to new Eppendorf tubes while the pellets were discarded. The total chlorophyll content, dissolved in the acetone, was determined by spectrophotometric analysis at 430 nm.

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Analysis of protein concentration: The protein analysis of the grown seeds after treatment with the various levels of NaF was determined through the Bradford assay. For this purpose, 0.5 g of plant tissues from each sample was homogenized in phosphate buffer saline. The homogenized tissues were then centrifuged at 10,000 rpm for 15 minutes at 4ºC. The proteins were precipitated by discarding the supernatant, adding of 1 mL of trichloroacetic acid to the pellet, and centrifuging at 5,000 rpm. Afterwards, the pellets were first washed with 80% acetone, to remove the traces of chlorophyll, and then washed again with distilled water. The precipitated proteins were dissolved by adding 1 mL of NaOH solution to all the tubes and the protein content was analyzed at 595 nm in a spectrophotometer.

Statistical examination: The information from all the NaF-treated seedlings and the control seedlings was verified and then compared by statistical examination with ANOVA and SPSS software. For all the analyses, $p<0.05$ was considered significant.

RESULTS

Effect of NaF treatment on seed germination: All the seeds were tested for their germinating capability. Higher NaF levels decreased the germination process in the maize seeds (Figure 2). In the control group, the germination rate was 100%. Treatment with NaF decreased germination with a 90% decrease occurring with 50 mg/L NaF.

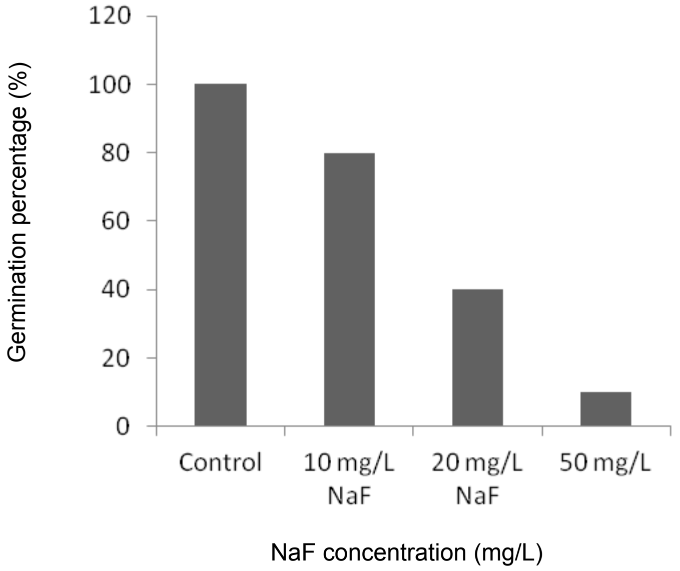

Figure 2. Total seed germination (%) of seedlings treated with NaF at concentrations of 0, 10, 20, and 50 mg/L.

Effect of NaF treatment on root and shoot length: The total root and shoot length decreased with increasing NaF concentrations (Figure 3). With 10 and 20 mg/L of NaF, the reductions were 30 and 40%, respectively. With 50 mg/L NaF, the total lengthwise measurement reduction was 70%.

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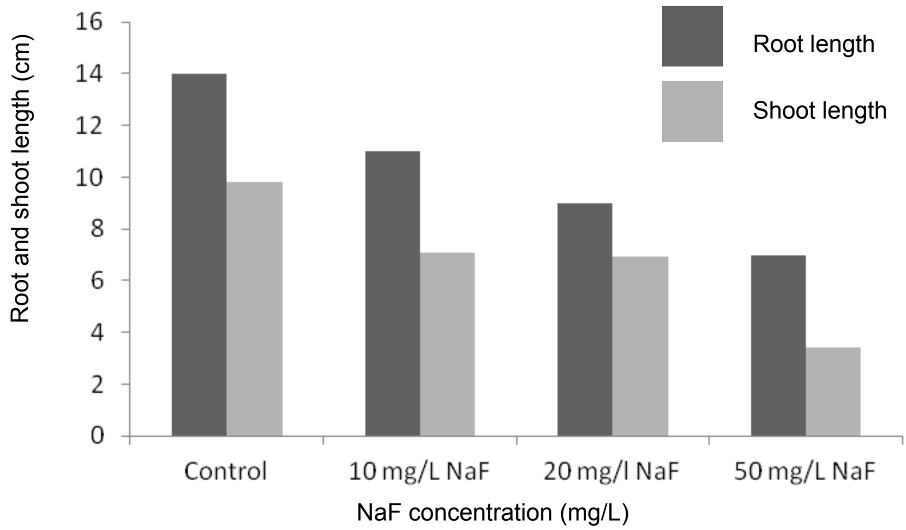

Figure 3. Root and shoot length (cm) of seedlings treated with NaF at concentrations of 0, 10, 20, and 50 mg/L.

Effect of NaF treatment on vigor index: The vigor index [(root length in cm + shoot length in cm) × germination percentage] decreased as the concentration of NaF was increased (Figure 4).

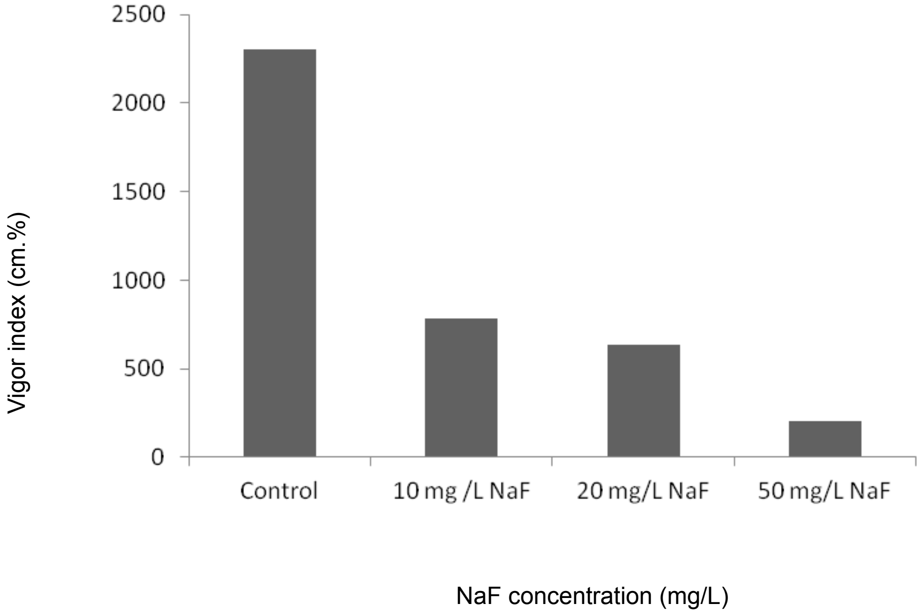

Figure 4. The vigor index (cm.%) of seedlings treated with NaF at concentrations of 0, 10, 20, and 50 mg/L. The root length and shoot length were measured in cm. The germination rate was measured as a percentage. The vigor index was calculated according to the equation:

Vigor index = (Root length [cm] + Shoot length [cm]) × Germination percentage (%)

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Effect of NaF treatment on total chlorophyll content: Treatment with NaF increased the total chlorophyll content with the peak occurring with 20 mg/L (Figure 5).

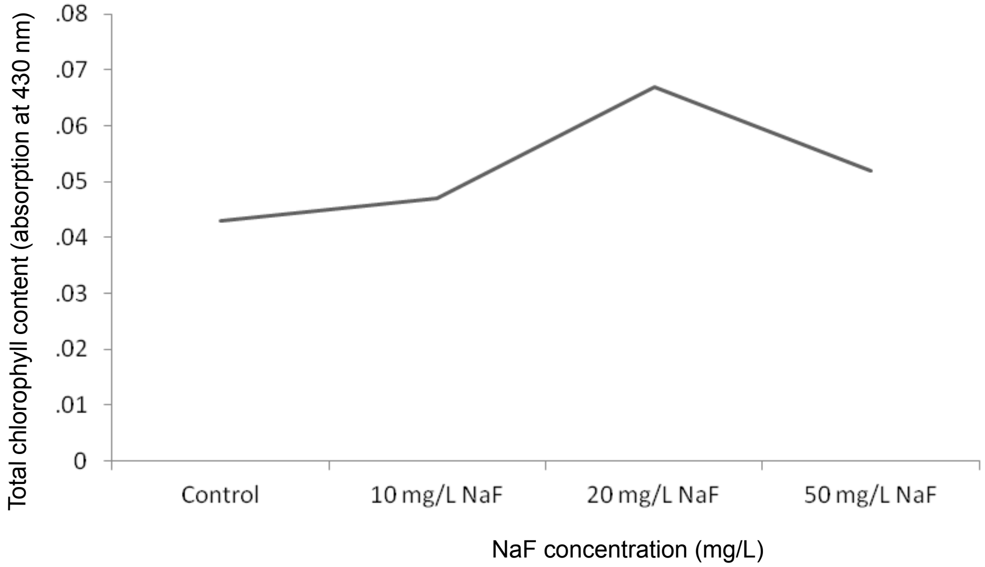

Figure 5. Total chlorophyll concentration of the leaves of maize seedlings, measured by absorption at 430 nm, treated with NaF at concentrations of 0, 10, 20, and 50 mg/L.

Effect of NaF treatment on total protein concentration: Increasing the NaF concentration resulted in a decrease in the total protein concentration in the seedlings (Figure 6).

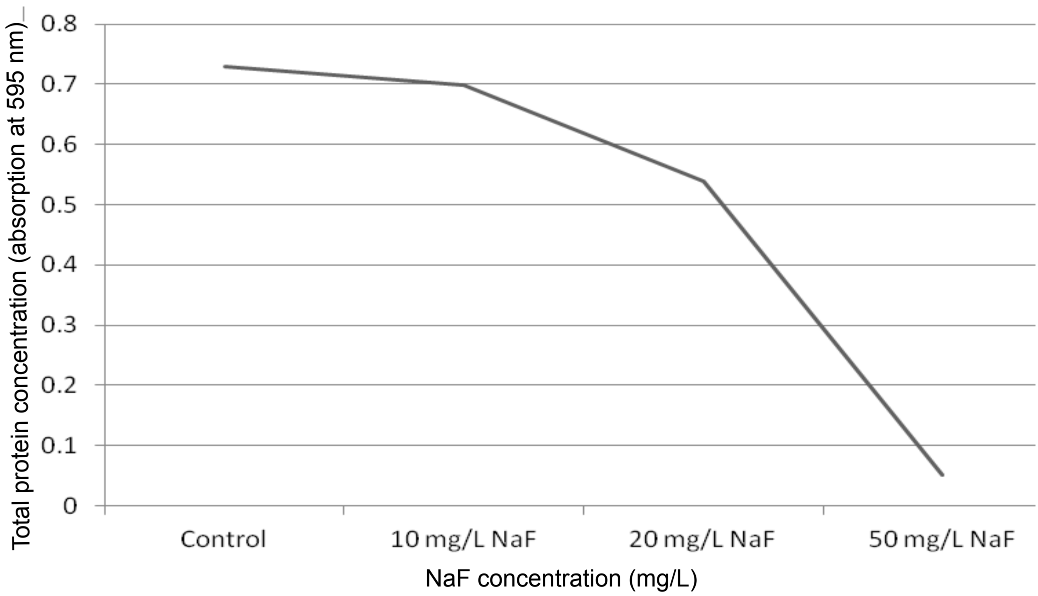

Figure 6. Total protein concentration of the maize seedlings, measured by absorption at 595 nm, treated with NaF at concentrations of 0, 10, 20, and 50 mg/L.

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DISCUSSION

The ion of the element fluorine, fluoride, is considered to be harmful for most plants. In response to its harmful impact on crop productivity, numerous researchers have demonstrated the negative effect of NaF on many important plants.[4] In the present study, we observed that as the concentrations of NaF were increased, there were adverse effects on various physical and chemical factors and ultimately reduced growth of the plants.

Seed germination was reduced with increasing levels of NaF due to an effect on some of the initial processes in seed germination. Weinstein et al. found NaF may inhibit the carbohydrate metabolism of germinating seeds.[7] It has been suggested the fluoride uptake reduces the germination by inhibiting the amylase activity which is essential for the germination process.[3]

Fluoride also affects the growth parameters such as the vigor index and the length of the plant roots and shoots by impairing the absorption of the nutrients and minerals absorption vital for normal plant development. Our finding of a progressively more adverse effect on root and shoot length with increasing concentrations of NaF is consistent with previous research.[13]

In the literature it is noted that fluoride stress reduces the pigment content of chlorophyll-a, chlorophyll-b, total chlorophyll, and carotenoids.[3] The possible causes for this are an increased breakdown of chlorophyll, the inhibition of chlorophyll biosynthesis, a stress-induced increase in the activity of the chlorophyll degrading enzyme chlorophyllase, and a fluoride-induced reduction in Fe^{+2} which is essential for chlorophyll synthesis.[3] In the present study, we found that the total chlorophyll content increased with the NaF treatment reaching a peak at 20 mg/L before falling to a lower level at 50 mg/L which was still above the control value. This findings are inconsistent with those in the literature and we do not have an explanation for this anomaly.

Fluoride also causes adverse effects on protein content of plants, particularly at higher concentrations. In the present study we found that increasing NaF concentrations decreased the total protein concentration, probably due to the disturbance of various metabolic processes including protein metabolism.[4]

CONCLUSION

Fluoride in concentrations of 10, 20, and 50 mg/L adversely affects the germination, growth, and total protein content of maize (*Zea mays* L.). Because fluoride can adversely affect the crop productivity of *Zea mays* L. it is recommended that the passage of fluoride into soil and water should be minimized as far as possible.

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