**SUMMARY:** Interactive effects of fluoride (F) and aluminium (Al) toward four species of microalgae at pH 7.3, 6.0, and 4.5, along with the accumulation of these elements by the algal cells, were the focus of this investigation. The species studied were *Scenedesmus obliquus*, *Microcystis aeruginosa*, *Anabaena sphaerica*, and *Nitzschia linearis*. At a concentration of 4 mg F/L, essentially no toxic effects were observed in any of the algal species at the three different pH values. The toxicity of Al, however, increased with decreasing pH. Interestingly, the combination of F + Al significantly ameliorated the toxic effect of Al at pH 6.0 toward *Scenedesmus* and *Microcystis*. With some dependence on pH, accumulation of Al was greater in *Microcystis* and *Nitzschia*, whereas accumulation of F was greater in *Scenedesmus* and about the same in *Anabaena*.

Keywords: Aluminum accumulation; Fluoride accumulation; Microalgae; Phytoplankton; Phytoplankton tolerance to Al and F.

**INTRODUCTION**

The continuing growth in global population has greatly increased the demand for freshwater. Human activities alter water quality not only by changing hydrologic pathways but also by the addition of substances and wastes to the landscape.\(^1\)

Aluminium is an abundant element in the earth’s crust, constituting about 8% of the soil minerals. Aluminium is present in many manufactured foods and medicines and is added to drinking water to remove turbidity. It has been proposed that aluminium is a contributing factor to several neurodegenerative disorders such as Alzheimer’s disease. However, this view remains controversial primarily because of the unusual properties of aluminium and a lack of information concerning its cellular sites of action.\(^2\)

Fluorine (F) occurs in natural waters as the fluoride ion (F\(^-\)), undissociated hydrofluoric acid (HF), and as various complexes. It is also known to inhibit a large number of biological processes including photosynthesis, respiration, protein synthesis, and enzyme activities of higher plants,\(^3\) green algae, cyanobacteria, and bacteria,\(^4^–^6\) at levels encountered from industrial fluoride pollution, but generally not at levels encountered in municipal fluoridation or in seawater.\(^7\)

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Since phytoplankton is primary producers of organic compounds in aquatic systems, there is a need to determine the interactive effects of Al and F on different species of microalgae. Although some reports have indicated that fluoride can ameliorate aluminium toxicity by the formation of fluoride-aluminium complexes, other studies suggest that fluoride can act synergistically to enhance aluminium toxicity. The purpose of this research was to investigate the interactive effect of Al and F toward several isolated species of microalgae at different pH values and to measure the accumulation of these elements in them.

MATERIALS AND METHODS

Organisms and growth conditions: Four microalgal strains were isolated from the phytoplankton community of the River Nile: \textit{Scenedesmus obliquus} (green algae), \textit{Microcystis aeruginosa} (colonial, blue-green algae), \textit{Anabaena sphaerica} (filamentous and heterocysts blue-green algae), and \textit{Nitzschia linearis} (diatoms). These organisms were grown in BG$_{11}$ containing (g/L): NaNO$_3$ (1.5); K$_2$HPO$_4$ (0.04); MgSO$_4$·7H$_2$O (0.075); CaCl$_2$ (0.036); citric acid (0.006); Na$_2$CO$_3$ (0.02); Na$_2$EDTA (0.001); and ferric ammonium citrate (0.006); plus minor elements (µg/L) added to 1.0 L of the BG$_{11}$ solution as 1 mL containing (g/L): H$_3$BO$_2$ (2.86); MnCl$_2$·4H$_2$O (1.81); ZnSO$_4$·7H$_2$O (0.222); Na$_2$MoO$_4$·2H$_2$O (0.39); CuSO$_4$·5H$_2$O (0.097); and Co(NO$_3$)$_2$·6H$_2$O (0.0494). Some modifications were made according to the type of species, e.g., in the case of \textit{Scenedesmus obliquus}, the sodium nitrate content was reduced to 1/5 the above amount, while in the case of \textit{Anabaena sphaerica} it was omitted from the media. Also, in the case of \textit{Nitzschia linearis}, 0.05 mg/L of Na$_2$SiO$_3$·5H$_2$O was included.

All strains were grown at optimum temperature (24 ± 2 ºC) with continuous illumination by white fluorescent lamps of ~2500 Lux. The stock cultures were continuously recultivated under optimum growth conditions and introduced into the experimental systems at logarithmic phase. Initial equivalent chlorophyll “a” concentrations for all experiments ranged from 25-30 µg/L. Bioassay flasks (conical 1-L capacity containing 500 mL of algal media) were incubated under optimum growth conditions. Flasks (in triplicate series) were shaken once each day to prevent clumping of the cells. Each experiment was run for 10 to 14 days to allow good growth without causing nutrient shortages. Growth in the cultures was determined by daily measurements of equivalent chlorophyll “a” content. At the end of each experiment, the algal mass was collected to determine fluoride and aluminium accumulation. The results presented are the averages of three experiments.

Stock solutions of sodium fluoride (NaF) and aluminium sulphate (Al$_2$(SO$_4$)$_3$·16H$_2$O) (ANALAR) were prepared before use to supplement
the culture media. Final fluoride and aluminium dose levels of 4 mg/L, which were found to be non-inhibitory at pH 7.3 in the BG11 media, were achieved by using stock solutions containing 1 mg F or Al/mL. All experiments were performed in triplicate at pH 7.3, 6.0, and 4.5 and repeated at least twice to verify reproducibility.

Fluoride and aluminium accumulation: To determine the amount of fluoride and aluminium accumulated by the cells, the algal mass at the end of each experiment was collected by centrifugation and washed three times with distilled water. The algal cells were dried at 105 °C (5 g after dryness) and then digested for Al and F accumulation according to APHA. The SPADNS colorimetric method was used for F determination, and the Eriochrome Cyanine R method was used for Al determination.

RESULTS

Response of the blue-green algae species to fluoride and aluminium: Figure 1 shows that the growth of *Microcystis aeruginosa* was not appreciably affected by the 4 mg/L dose of F at different pHs. However, the Al toxicity to *Microcystis* was greatly increased with decreasing pH of the media, the percentage reduction in algal biomass being 49, 98, and 98% at pH 7.3, 6.0, and 4.5, respectively. At pH 6.0, but not at pH 4.5, F at 4 mg/L ameliorated the toxicity effect of Al to *Microcystis*.

![Figure 1. Response of blue-green alga (*Microcystis aeruginosa*) to fluoride and aluminium at different pHs.](image)

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In the case of the blue-green alga *Anabaena sphaerica*, the most conspicuous result was that decreasing pH has an injurious effect on the alga growth (Figure 2). The maximum algal biomass in the control culture at pH 7.3 was 820 µg/L of chlorophyll “a” equivalent, while at pH 4.5 it was only 64 µg/L. Chlorophyll “a” equivalent in the control culture at pH 4.5. The ameliorating effect of F toward Al toward the algal growth was observed at pH 7.3 and 6.0, with only a slight difference between the effect of Al alone or in combination-with F.

Response of the green alga to fluoride and aluminium: The response of *Scenedesmus obliquus* towards fluoride and aluminium alone or in combination is shown in Figure 3. On treatment with 4 mg F/L, slight inhibition can be detected at decreasing pH. The toxicity of Al, however, increased greatly with decreasing pH. In addition, the ameliorating effect of F towards Al toxicity under all three pH conditions was readily apparent.

Response of the diatoms alga to fluoride and aluminium: As in the *Anabaena sphaerica* culture (Figure 2), an acidity of pH 4.5 has a pronounced injurious effect on the growth of *Nitzschia linearis* in the control culture (Figure 4). At the same time, the presence of added fluoride has essentially no ameliorating effect on the toxicity of Al to this alga.

Fluoride and aluminium accumulation: As seen in Figure 5, the algal species studied here differ greatly in their ability to take up and accumulate fluoride and aluminium. The blue-green species (*Microcystis aeruginosa* and *Anabaena sphaerica*) tended to accumulate more F in their cells than the other
two species. In the same time, *Nitzschia linearis* (diatoms) has the ability to accumulate Al more than the other species of algae.

**Figure 3.** Response of green alga (*Scenedesmus obliquus*) to fluoride and aluminium at different pHs.

**Figure 4.** Response of diatoms (*Nitzschia linearis*) to fluoride and aluminium at different pHs.
Figure 5. Fluoride and aluminium accumulation inside the algal cells
DISCUSSION
The continuing rapid growth in the requirement for potable water, has increased the importance for studying and understanding the interactions between chemical substances used in the treatment of drinking water. In the present study, the most important results are that species belonging to blue-green algae differ between each other in their tolerance to tested dose levels of F and Al at various pHs. The results also showed that the green algae can tolerate and are fairly resistant to the toxicity of F and Al at different pHs with the percentage reduction in algal biomass increasing with increasing acidity of the algal culture. This dependence of toxicity on pH may be a consequence of pH-transformed chemical forms of fluoride and aluminium in solution.13-17
In addition, it has been hypothesized that F transport through biological membranes occurs primarily through non-ionic diffusion of HF which increases at the acidic pH. Thus, it is the HF concentration and not the total quantity of fluoride that governs toxicity.

The results of this study also support the hypothesis that aluminium toxicity against the green alga (Scenedesmus obliquus) is enhanced in an acidic environment.17-19 This can also be seen in the growth inhibition of Nitzschia linearis at pH 4.5, which may be attributed to the acidity rather than to the toxic effect of fluoride or aluminium.

On the other hand, a change in pH plays an important role in the toxicity of Al, especially in the presence of F, where it can form complexing ligands. The formation of AlF$_4^-$, being a PO$_4^{3-}$ analogue, might compete with PO$_4^{3-}$ for binding sites of ATPase. Inhibition of ATPase affects the uptake of vital ions including NO$_3^-$ and PO$_4^{3-}$. Therefore, a reduced nutrient uptake in the presence of test chemicals at acidic pH seems to be a major cause of inhibition of growth.8

The amounts of Al and F accumulated in the algal cells show that different species of algae differ in their ability to accumulate F and Al inside their cells. The accumulation rate depends on the type of algae,17-23 since Nichol et al23 isolated both resistant and tolerant forms of Synechococcus leopoliensis and observed that resistant cells show passive permeation of both F$^-$ and HF across the cell membrane. The sensitive cells are permeable only to HF and thus accumulate fluoride to a toxic concentration.

REFERENCES