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# Interactive Effects of Aluminum and Humic Substances on Salvinia

JEFFREY L. GARDNER AND SAFAA H. AL-HAMDANI[1]

## ABSTRACT

Salvinia increased the pH of the growth media within two days to near neutral pH in the absence of Al with and without humic substances. In most cases, Al in the absence of humic substances reduced salvinia growth, chlorophyll *a* and *b* concentrations, and carotenoid concentrations. Reductions were greater with increasing concentrations of Al and humic substances alleviated some of the toxic effects of Al. Also, corrected treatments (pH 3.9) influenced an increase in the above parameters for most treatments compared to uncorrected treatments (pH varied according to the treatment). Anthocyanin concentrations of salvinia increased in treatments receiving Al. The accumulation of soluble sugars, starch, and total nonstructural carbohydrates (TNC) increased in the presence of 20.0 mg/l Al without humic substances and decreased in treatments receiving humic substances.

*Key words:* aquatic plant, metal toxicity, pH, plant pigment, carbohydrate.

[1]Graduate Assistant and Assistant Professor, respectively, Biology Department, Jacksonville State University, Jacksonville, AL 36265. Received for publication September 6, 1996 and in revised form January 10, 1997.

## INTRODUCTION

Salvinia (*Salvinia minima* Willd.) is a small, free-floating freshwater fern found in tropical and temperate regions of the world (DeBusk and Reddy 1987). Under favorable conditions these plants spread by vegetative reproduction and can colonize large areas of water in short periods of time (Oliver 1993). Sale et al. (1985) suggested that explosive growth rates were possibly due to community photosynthesis related to a high rate of photosynthetic efficiency.

Aluminum (Al) finds its way into aquatic ecosystems by agricultural, manufacturing, mining, and waste disposal practices (Foy et al. 1978). Aluminum is the most abundant metal in the earth's crust; however, the Al concentration in fresh water varies according to the location (Poleo 1995). Aluminum was shown to interfere with the uptake, transport, and use of Ca, Mg, P, and K (Foy et al. 1978). Reuter et al. (1987) reported that 60 µM of Al was required to produce significant inhibitory effects of *Scenedesmus*. Aluminum was also reported to be the principle toxicant killing fish due to surface acidification of natural waters in the northern temperate regions (Poleo 1995).

The toxicity of Al depends upon many factors, including water pH and organic matter content (Tan and Binger

1986). Aluminum toxicity has been known to increase at a pH < 5 because its solubility increases. Of the different species of Al, $Al^{3+}$ is the only significant form that is found in freshwater at a pH of 4 or less (Poleo 1995). Buckler et al. (1995) reported that as the water acidity changes, Al changes from an Al hydroxyl ion at pH 7.2 to various Al hydroxide monomers, polymers, and free Al at a lower pH. In nature, Al is ubiquitous in most freshwater and will most likely exist in the presence of decomposed organic matter. Dissolved humic substances comprise the major portion of dissolved organic materials and typically range from 1 to 70 mg/l (Stackhouse and Benson 1989). Freshwater humic, usually composed of 90% fulvic acid and 10% humic acid, was reported to reduce the sensitivity of *Chlamydomonas reinhardtii* to Cu (Garvey et al. 1991). Poleo (1995) suggested that humic acid reduced Al toxicity to fish by inhibiting Al polymerization. Guilizzoni (1991) reported that complexation is one of the most important factors in reducing the toxicity of heavy metals in aquatic systems. Stackhouse and Benson (1989) reported that 50 mg/l humic acid decreased the bioaccumulation of Cd in *Daphnia magna* and proposed that humic acid more readily binds to cations rather than anions. Most of the published toxicity studies disregard the fact that humic substances are a natural constituent of freshwater through the decomposition of plant organic material. Furthermore, in those toxicity studies where humic substances were considered, commercially available humic acid extracted from terrestrial sources was used (Garvey et al. 1991, Zitko et al. 1973, and Winner 1985). In fact, there is very limited information available on the interactive effects of commercially or naturally extracted humic substances and Al on aquatic freshwater plants in general and especially on salvinia. Therefore, the objectives of this investigation were to determine the interactive effects of different concentrations of Al (10.0 and 20.0 mg/l) and naturally extracted humic substances of common cattail (*Typha latifolia* L.) on salvinia growth, selected pigments, and carbohydrate accumulation. Acidification of aquatic ecosystems was established as a contributing factor in the reduction of acid-intolerant aquatic plants such as *Ranunculus peltatus* Schrank and *Myriophyllum alterniflorum* DC. (Maessen et al. 1992) and a decline in natural fish populations (Buckler et al. 1995). Consequently, an additional objective of this study was to examine the ability of salvinia in buffering its environment under the above experimental conditions and possibly correct some of the negative impact which is usually associated with low pH.

## MATERIAL AND METHODS

The humic material was obtained from leaves of common cattail, which was allowed to decompose for approximately one year under greenhouse conditions. The humic substances were collected and purified according to the procedure of Wilson and Al-Hamdani (1997). The filtrate was diluted with distilled water to obtain an absorption of 0.10 at 250 nm. The selected wavelength was observed to be the peak absorbance for the humic substances among a wide range of examined wavelengths.

Salvinia plants with a total of 30 leaves were placed in each of 12 Erlenmeyer flasks representing each treatment. Each flask contained 125 ml of modified Hoagland solution (Hoagland and Arnon 1938), diluted 1:40 (Sela et al. 1989) at pH 3.9.

All replicates of each treatment received concentrations of 0.0, 10.0, or 20.0 mg/l Al with or without humic substances. Aluminum was supplied in the form of $AlK(SO_4)_2 \times 12H_2O$ which was added to the growth media to obtain the above concentrations. The plants were placed in a growth chamber at 25C, 220 $\mu$mol $m^{-2}s^{-1}$ photon flux density, and a 14-h photoperiod.

Salvinia was grown under the above conditions for 14 days with the growth solution being changed after seven days to minimize algae contamination. At the end of 14 days, six randomly selected samples of 12 total samples of each treatment were used for dry weight and carbohydrate determination. The other samples were used to test for chlorophyll *a* and *b*, carotenoid and anthocyanin concentrations.

Twelve replicates of each treatment were used to determine changes in the pH of the growth media. The pH of each sample was recorded every two days using a Fisher Accumet pH Meter (Model 610A). The pH for six replicates of each treatment were readjusted (corrected) to 3.9 by adding 0.01 N HCl while the other six replicates were left unadjusted (uncorrected).

The plants of six samples of each treatment were oven dried for 2-h at 80C to stop enzyme action, then dried for 48-h at 70C. The dry weights of each sample were recorded and stored at -20C for subsequent determination of carbohydrates.

A sample of 0.10 g fresh weight was placed in a 10 ml vial containing 5 ml of N,N-Dimethylformamide (DMF) and incubated in the dark for 36-h at 4C in order to extract chlorophyll. Chlorophyll *a* and *b* content was determined spectrophotometrically by the method of Inskeep and Bloom (1985). The carotenoid concentration was determined spectrophotometrically from the N,N-DMF extraction and total concentration was calculated using the formula of Doong et al. (1993). In addition, anthocyanin concentration was determined following the method of Mancinelli (1990).

Carbohydrate analysis of the plant samples was conducted following a slightly modified procedure from that outlined by Chatterton et al. (1987). The samples were ground into a fine powder and a 100-500 mg portion was placed in a sealed vial and used for the determination of soluble sugars, starch, and total nonstructural carbohydrates (TNC) as reported in detail by Wilson and Al-Hamdani (1997).

Each of these experiments were repeated twice and statistically analyzed as a randomized complete design (Steel and Torrie 1980). The design ensured that observed differences in the plants performance were due to treatments rather than variations among blocks (replicate series conducted at different times). Mean separations for the values which were shown significant F values ($P < 0.05$) of the ANOVA analysis were based on the least significant difference (LSD) test (Steel and Torrie 1980).

## RESULTS AND DISCUSSION

Control plants grown in the absence of humic substances and Al resulted in an increase in the initial pH of the growth media after two days (Figure 1). The pH continued to increase from 3.9 to 6.9 at day two to 7.4 at day six. Even though the pH of the growth media was readjusted to 3.9 on day seven, the pH increased to 7.9 within one day. After day eight, the pH gradually decreased to 6.7 at the conclusion of the experiment. However, the pH of the growth media containing 10.0 and 20.0 mg/l Al remained acidic throughout the experiment. The pH of the growth media containing both 20.0 mg/l Al and humic substances increased to 5.4 within six days. In contrast, the treatment containing 10.0 mg/l Al with humic substances resulted in an increase in pH within four days. During the second week, the pH increased to 5.7 within one day of readjustment of the media containing 10.0 mg/l Al with humic substances. Humic substances in the absence of Al had a similar effect on the pH to that shown for the control plants (Figure 1).

Aluminum toxicity is closely related to pH and is considered to be most toxic to plants at a pH below 5 (Haug 1984). The ability of salvinia to buffer the growth media, in most cases, increased the pH above the level that Al is most toxic (Figure 1). The ability of some aquatic plants to adjust the pH of their environment is a well known phenomena (Haller and Sutton 1973, Vu et al. 1986). In general, plants growing in the presence of humic substances with Al were able to increase the pH to a higher level in comparison to those treatments receiving Al without humic substances (Figure 1). This indicates that the humic substances could possibly chelate some of the $H^+$ and reduce its concentration.

The presence of 10.0 and 20.0 mg/l Al without humic substances in the growth media resulted in a reduction in salvinia growth at both the corrected and the uncorrected pH treatments (Table 1). This reduction in the plants growth was even greater with those grown at 20.0 mg/l in comparison to those grown at 10.0 mg/l. The presence of Al might

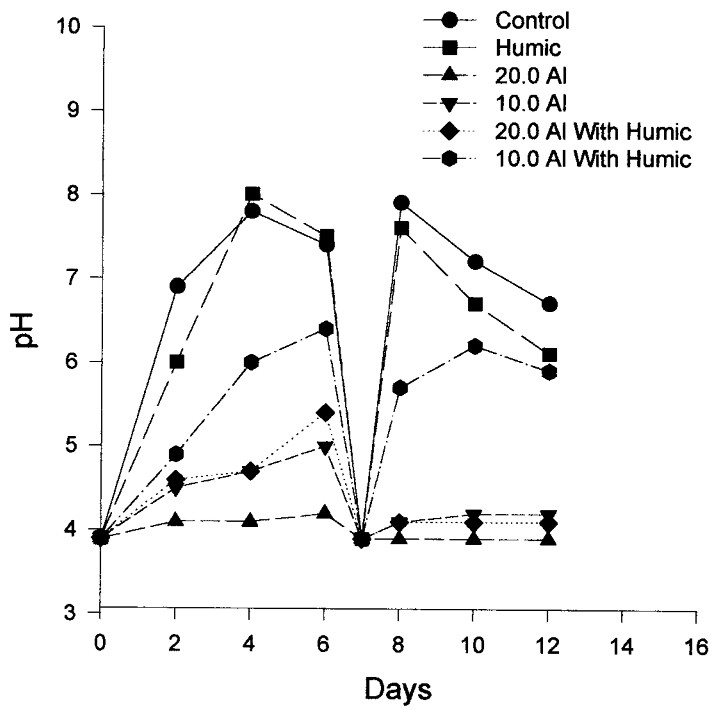


Figure 1. Interactive effects of Al (mg/l) and humic substances on pH of growth media for salvinia. The initial pH of the growth media was 3.9. The growth media was changed after one week and the pH was readjusted to 3.9.

Table 1. Interactive effects of Al (mg/l) and humic substances on salvinia growth (mg Dry Weight). The initial pH of the growth media was 3.9. The pH was readjusted every two days to 3.9 for the corrected treatments.

| Treatment | Corrected pH* | Uncorrected pH |
|---|---|---|
| Control | 62.8a | 62.1a |
| Humic | 60.3a | 57.8a |
| 20.0 Al | 30.7b | 29.4b |
| 10.0 Al | 47.3c | 42.4c |
| 20.0 Al with Humic | 42.8d | 51.6d |
| 10.0 Al with Humic | 50.4e | 59.0e |

*Values for plant dry weight in a column followed by the same letter are not significantly different at the 0.05 level according to LSD test.

cause a decrease in the uptake and transport of some of the essential plant nutrients. Maessen et al. (1992) reported that elevated levels of Al inhibited the uptake of Ca, Mg, Fe, Mn, and Zn by competing for binding sites. Furthermore, Al was shown to interfere with mitosis and cell elongation in the root system of *Allium ursinum* L. (Andersson 1993). However, with the exception to that obtained at 10.0 mg/l Al with humic substances for the corrected media, the presence of Al with humic substances resulted in an increase in salvinia growth in comparison to those plants grown at 10.0 and 20.0 mg/l Al in the absence of humic substances (Table 1). Rueter et al. (1987) reported that naturally occurring humic acid can bind aluminum competitively and reduce its toxic effect. In addition, DeFilippis and Pallaghy (1994) suggested that bioaccumulation of a metal is a function of its ionic level in the growth media. The ionic level of Al was probably reduced by possibly binding to the humic substances, thus limiting its uptake by the plant. Tan and Binger (1986) reported that organic matter not only decreased Al solubility, but also lowered the critical value at which plants were injured by acidity. Furthermore, Hue et al. (1986) suggested that the detoxifying capacity of organic chelating agents, such as humic substances, is related to the relative positions of OH/COOH groups on the main carbon chain. In this study, humic substances in the absence of Al insignificantly influenced plant growth. However, corrected treatments containing Al and humic substances were shown to decrease plant growth in comparison to that obtained with the uncorrected treatments.

Chlorophyll *a* and *b* concentrations of salvinia decreased in the presence of Al at both 10.0 and 20.0 mg/l for the corrected and uncorrected treatments (Table 2). The reduction was greater at 20.0 mg/l in comparison to those at 10.0 mg/l. This decline in chlorophyll concentrations might be caused by a reduction in the synthesis of chlorophyll by possibly increasing chlorophyllase activity (Sen et al. 1987). Furthermore, Pettersson et al. (1985) suggested that a reduction in chlorophyll concentrations of *Anabaena cylindrica* treated with Al might be caused by an ultrastructural change in the membrane. The chlorophyll *a* and *b* concentrations were similar in the corrected and uncorrected treatments (Table 2). However, both the corrected control and the corrected treatment containing 20.0 mg/l Al with humic substances resulted in increasing the chlorophyll *a* and *b* concentrations in comparison to the corresponding uncorrected treatments.

Table 2. Interactive effects of Al (mg/l) and humic substances on salvinia chlorophyll (mg/g Fresh Weight). The initial pH of the growth media was 3.9. The pH was readjusted every two days to 3.9 for the corrected treatments.

| | Corrected pH* | | Uncorrected pH | |
|---|---|---|---|---|
| Treatment | chl a | chl b | chl a | chl b |
| Control | 5.7a | 4.1a | 4.7a | 3.3a |
| Humic | 4.9b | 3.5b | 4.5a | 3.1a |
| 20.0 Al | 4.6c | 1.6c | 2.5b | 1.6b |
| 10.0 Al | 3.7d | 2.5d | 3.2c | 2.0b |
| 20.0 Al with Humic | 3.5d | 2.3d | 2.8bc | 1.7b |
| 10.0 Al with Humic | 3.6d | 2.3d | 3.2c | 2.1b |

*Values for chlorophyll a and b in a column followed by the same letter are not significantly different at the 0.05 level according to LSD test.

Furthermore, chlorophyll *a* and *b* concentrations were insignificantly effected in uncorrected treatments receiving humic substances in the absence of Al and significantly decreased in corrected treatments.

Carotenoid concentrations decreased in the presence of 10.0 and 20.0 mg/l Al in the growth media (Table 3). The reductions in carotenoids might be caused by swelling of the chloroplasts or possibly by thylakoid disarrangement (Bassi et al. 1990). With the exception of the corrected treatment containing 20.0 mg/l Al and humic substances, the presence of humic substances in treatments containing Al insignificantly effected carotenoid concentrations in comparison to that obtained for the same Al concentration in the absence of humic substances. As with plant growth and chlorophyll concentrations, carotenoids were reduced in treatments containing humic substances without Al. This implies that humic substances might form complexes with essential nutrients. Kim and Wetzel (1993) reported similar findings with *Anabaena flos-aquae* at 10.0 mg/l humic acid. Corrected treatments influenced a reduction in the toxic effects of Al which resulted in increased carotenoid concentrations.

Doong et al. (1993) reported that anthocyanins are produced by aquatic plants in response to stress factors. In this study, the anthocyanin content of salvinia increased in the presence of Al without humic substances for both the corrected and uncorrected treatments (Table 3). In general, the presence of humic substances did not interfere with Al influence on anthocyanin content. Corrected treatments, with the exception of the control and 10.0 mg/l Al with humic substances, resulted in an increase in anthocyanin content. Haug (1984) suggested that anthocyanins are capable of chelating Al. This indicates that salvinia increased anthocyanin synthesis as a mechanism of detoxifying Al.

The accumulation of soluble sugars in salvinia were insignificantly effected by the presence of 20.0 and 10.0 mg/l Al for the uncorrected treatments (Table 4). However, at 20.0 mg/l Al for the corrected treatment, an increase in soluble sugars was obtained in comparison to the corrected control. The presence of humic substances with 20.0 mg/l Al for the corrected and uncorrected treatments influenced a decrease in soluble sugars compared to 20.0 mg/l in the absence of humic substances. Starch accumulation increased in the presence of 20.0 mg/l Al without humic substances for both the corrected and uncorrected treatments in comparison to the controls (Table 4). The presence of humic substances with Al influenced a reduction in starch accumulation compared to the same Al concentration without humic substances for all treatments except for the corrected treatment containing 10.0 mg/l Al with humic substances. Total nonstructural carbohydrates increased for both the corrected and uncorrected treatments at 20.0 mg/l Al in the absence of humic substances (Table 4). However, at 10.0 mg/l Al without humic substances, no change in TNC was obtained. The addition of humic substances with 20.0 mg/l Al decreased TNC compared to the corresponding Al concentrations in the absence of humic substances for both the corrected and uncorrected treatments. Corrected treatments in the presence of Al with or without humic substances influenced an increase in TNC in comparison to the uncorrected treatments. This should not be interpreted as though Al induced an increase in carbohydrate synthesis. Rather it most likely influenced a reduction in carbohydrate utilization. Schroll (1978) observed that metal toxicity resulted in a less inhibitory effect to photosynthesis than to overall plant growth. Furthermore, Roy et al. (1988) reported that Al decreased respiration and carbohydrate utilization. Azcon-Bieto (1983) suggested that soluble sugar accumulation might reduce RuBP regeneration by decreasing available stromal $P_i$. In addition, Roy et al. (1988) reported that Al binds to phosphate compounds, thus reducing the availability of phosphates.

It is clear that this study showed that Salvinia is capable of buffering its environment and increased the pH above the

Table 3. Interactive effects of Al (mg/l) and humic substances on salvinia carotenoids and anthocyanins (mg/g Fresh Weight). The initial pH of the growth media was 3.9. The pH was readjusted every two days to 3.9 for the corrected treatments.

| | Corrected pH* | | Uncorrected pH | |
|---|---|---|---|---|
| Treatment | Carotenoids | Anthocyanins | Carotenoids | Anthocyanins |
| Control | 96.2a | 1.2a | 67.3a | 1.2ac |
| Humic | 77.3b | 1.4ab | 58.8a | 0.8a |
| 20.0 Al | 21.2c | 1.8bc | 19.9b | 1.7b |
| 10.0 Al | 39.1d | 1.7bc | 28.0b | 1.6b |
| 20.0 Al with Humic | 33.9d | 1.9c | 23.7b | 1.7b |
| 10.0 Al with Humic | 32.7d | 1.3a | 30.2b | 1.4bc |

*Values for carotenoids and anthocyanins in a column followed by the same letter are not significantly different at the 0.05 level according to LSD test.

TABLE 4. INTERACTIVE EFFECTS OF AL (MG/L) AND HUMIC SUBSTANCES ON SALVINIA CARBOHYDRATE ACCUMULATION (MG/G DRY WEIGHT). THE INITIAL PH OF THE GROWTH MEDIA WAS 3.9. THE PH WAS READJUSTED EVERY TWO DAYS TO 3.9 FOR THE CORRECTED TREATMENTS.

| | Corrected pH* | | | Uncorrected pH | | |
|---|---|---|---|---|---|---|
| Treatment | Soluble Sugars | Starch | TNC | Soluble Sugars | Starch | TNC |
| Control | 9.9ac | 138.5a | 160.0a | 12.2a | 148.5ace | 173.3a |
| Humic | 8.5a | 139.9a | 161.1a | 7.6b | 144.0ade | 163.8ac |
| 20.0 Al | 13.6b | 196.1b | 224.1b | 13.0a | 193.0b | 219.7b |
| 10.0 Al | 9.1a | 158.4c | 179.3c | 10.0c | 154.0c | 174.5a |
| 20.0 Al with Humic | 9.5a | 174.8d | 196.8d | 9.1bc | 150.0cd | 169.1a |
| 10.0 Al with Humic | 11.0c | 159.4c | 179.6c | 8.2bc | 139.1e | 156.3c |

*Values for carbohydrates in a column followed by the same letter are not significantly different at the 0.05 level according to LSD test.

critical level that is considered to be optimum for Al toxicity. In most cases, the buffering capacity of Salvinia was reduced as well as plant growth at high Al concentrations. Plant growth, chlorophyll, and carotenoid concentrations were enhanced for corrected treatments in comparison to uncorrected treatments. This could be interpreted that salvinia responds favorably to a low pH environment.

## LITERATURE CITED


Andersson, M. E. 1993. Aluminum toxicity as a factor limiting the distribution of *Allium ursinum* (L.). Ann. Bot. 72: 607-611.

Azcon-Bieto, J. 1983. Inhibition of photosynthesis by carbohydrates in wheat leaves. Plant Physiol. 73: 681-686.

Bassi, M., M. G. Corradi, and A. Ricci. 1990. Effects of chromium (VI) on two freshwater plants, *Lemna minor* and *Pistia stratiotes*. 2 biochemical and physiological observations. Cytobios. 62: 101-109.

Buckler, D. R., L. Cleveland, E. E. Little, and W. G. Brumbaugh. 1995. Survival, sublethal responses, and tissue residues of Atlantic salmon exposed to acidic pH and aluminum. Aquat. Toxicol. 31: 203-216.

Chatterton, N. J., P. A. Harrison, J. H. Bennett and W. Thornley. 1987. Fructan, starch, and sucrose concentrations in crested wheatgrass and redtop as affected by temperature. Plant Physiol. Biochem. 25: 617-623.

DeBusk, W. F. and K. R. Reddy. 1987. Growth and nutrient uptake potential of *Azolla caroliniana* Willd. and *Salvinia rotundifolia* Willd. as a function of temperature. Environ. Exp. Bot. 27: 215-221.

DeFilippis, L. F. and C. K. Pallaghy. 1994. Heavy metals: sources and biological effects. In: Rai, L. C., Gaur, J. P. and Soeder, C. J. (Editors), Algae and Water Pollution. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, pp. 31-77.

Doong, R. L., G. E. MacDonald, and D. G. Shilling. 1993. Effect of fluridone on chlorophyll, carotenoid, and anthocyanin content of *Hydrilla*. J. Aquat. Plant Manage. 31: 55-59.

Foy, C. D., R. L. Chaney, and M. C. White. 1978. The physiology of metal toxicity in plants. Ann. Rev. Plant Physiol. 29: 511-566.

Garvey, J. E., H. A. Owen, and R. W. Winner. 1991. Toxicity of copper to the green alga, *Chlamydomonas reinhardtii* (Chlorophyceae), as affected by humic substances of terrestrial and freshwater origin. Aquat. Toxicol. 19: 89-96.

Guilizzoni, P. 1991. The role of heavy metals and toxic materials in the physiological ecology of submersed macrophytes. Aquat. Bot. 41: 87-109.

Haller, W. T. and D. L. Sutton. Effect of pH and high phosphorus concentrations on growth of waterhyacinth. Hyacinth Contr. J. 11: 59-61.

Haug, A. 1984. Molecular aspects of aluminum toxicity. CRC Crit. Rev. Plant Sci. 1: 345-373.

Hoagland, D. R. and D. I. Arnon. 1938. The water-culture method for growing plants without soil. Univ. Calif. Agri. Exp. Stn. Cir. No. 347. pp. 1-32.

Hue, N. V., G. R. Craddock, and F. Adams. 1986. Effect of organic acids on aluminum toxicity in subsoils. Soil Sci. Soc. Am. J. 50: 28-34.

Inskeep, W. P. and P. R. Bloom. 1985. Extinction coefficients of chlorophyll *a* and *b* in N, N- dimethylformamide and 80% acetone. Plant Physiol. 77: 483-485.

Kim, B. and R. G. Wetzel. 1993. The effect of dissolved humic substances on the alkaline phosphatase and the growth of microalgae. Verh. Internat. Verein. Limnol. 25: 129-132.

Maessen, M., J. G. M. Roelofs, M. J. S. Bellemakers, and G. M. Verheggen. 1992. The effects of aluminum, aluminum/calcium ratios and pH on aquatic plants from poorly buffered environments. Aquat. Bot. 43: 115-127.

Mancinelli, A. L. 1990. Interaction between light quality and light quantity in the photoregulation of anthocyanin production. Plant Physiol. 92: 1191-1195.

Oliver, J. D. 1993. A review of the biology of giant *Salvinia* (*Salvinia molesta* Mitchell). J. Aquat. Plant Manage. 31: 227-231.

Pettersson, A. L., L. Haellbom, and B. Bergman. 1985. Physiological and structural responses of the cyanobacterium *Anabaena cylindrica* to aluminum. Physiol. Plant. 63: 153-158.

Poleo, A. B. S. 1995. Aluminum polymerization-a mechanism of acute toxicity of aqueous aluminum to fish. Aquat. Toxicol. 31: 347-356.

Roy, A. K., A. Sharma, and G. Talukdar. 1988. Some aspects of aluminum toxicity in plants. Bot. Rev. 54: 145-178.

Rueter, J. G., K. T. O'Reilly, and R. R. Petersen. 1987. Indirect aluminum toxicity to the green alga *Scendesmus* through increased cupric ion activity. Environ. Sci. Technol. 21: 435-438.

Sale, P. J. M., P. T. Orr, G. S. Shell, and D. J. Erskine, D. J. C. 1985. Photosynthesis and growth rates in *Salvinia molesta* and *Eichhornia crassipes*. J. Appl. Ecol. 22: 125-137.

Schroll, H. 1978. Determination of the absorption of $Cr^{+6}$ and $Cr^{+3}$ in an algal culture of *Chlorella pyrenoidosa* using $^{15}Cr$. Bull. Environ. Contam. Toxicol. 20: 721-724.

Sela, M., J. Garty, and E. Tel-Or. 1989. The accumulation and the effect of heavy metals on the water fern *Azolla filiculoides*. New Phytol. 112: 7-12.

Sen, A. K., N. G. Mondal, and S. Mandal. 1987. Studies of uptake and toxic effects of Cr (VI) on *Pistia stratiotes*. Wat. Sci. Tech. 19: 119-127.

Stackhouse, R. A. and W. H. Benson. 1989. Interaction of humic acid with selected trace metals: Influence on bioaccumulation in Daphnids. Environ. Toxicol. Chem. 8: 639-644.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and procedures of statistics: a biometrical approach. 2nd ed. McGraw-Hill, New York, 196 pp.

Tan, K. H. and A. Binger. 1986. Effect of humic acid on aluminum toxicity in corn plants. Soil Sci. 141: 20-25.

Vu, V. V., H. W. Sang, J. W. Kijne, K. Planque, and R. Kraayenhof. Effects of temperature, pH and bound nitrogen on photosynthesis and nitrogen fixation of *Azolla pinnata* and *Azolla filiculoides*.

Wilson, J. G. and S. H. Al-Hamdani. 1997. Effects of Cr (VI) and humic substances on selected physiological responses of *Azolla caroliniana*. Am. Fern J. (In Press).

Winner, R. W. 1985. Bioaccumulation and toxicity of copper as affected by interactions between humic acid and water hardness. Wat. Res. 19: 449-455.

Zitko, P., W. V. Carson, and W. G. Carson. 1973. Prediction of incipient lethal levels of copper to juvenile Atlantic salmon in the presence of humic acid by cupric electrode. Bull. Environ. Contam. Toxicol. 10: 265-271.