Original Research Paper

Acute and Chronic Toxicity of Aluminium Fluoride to Flora and Fauna in a Microcosm

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ABSTRACT

During acute toxicity study of aluminium fluoride, *Daphnia similis* L. was found to be the most sensitive organism (EC_{50} = 108.06 ppm) followed by *Gambusia affinis* Baird and Gerard (LC_{50} = 354.0 ppm) and *Lemna aequinoctialis* L. (EC_{50} for chlorophyll = 358.7ppm). The exposure (60 days) of producers and consumers at its sub-lethal concentration (35.4 ppm) casted toxic effects on them in artificial microcosms raised in the greenhouse. There was reduction in chlorophyll content (19-39%), dry weight (16%), acid phosphatase (ACP) (56%), alkaline phosphatase (ALP) (14%) and protein content (53%) of *Ceratophyllum demersum* L. The reduction in species richness (40%) and phytoplankton counts (counts = 47-54%) was significant during the study period while zooplankton counts (30%) in the first half of the study (day-30). Snail mortality was found nil while that of fish was moderate (37%). Their tissue biochemistry (ACP, ALP and protein content) was, however, altered significantly suggesting them to be under stress. AlF_3 also had cytotoxic effects in fish decreasing RBC counts (19%) and causing morphological abnormalities. From these findings, we conclude that there are significant toxic effects of aluminium fluoride to organisms in the food web of aquatic ecosystems.

INTRODUCTION

The commercial applications of aluminium fluoride in the production of aluminium, ceramics, glass manufacturing and as a catalyst for organic synthesis and inhibitor of fermentation contribute this compound in the environment. Its other important source is widespread acid rains prevalent mostly in the temperate countries which led to most of aluminium found in acidic waters and soils (pH < 5) to bind with fluoride forming aluminium fluoride complexes.

Fluorine-aluminium complexes have received most attention in relation to soil (Bower & Hatcher 1967, Omueti & Jones 1977, Davison 1983) and, more recently, in connection with acidification of freshwater (Driscoll et al. 1980, Johnson et al. 1981). They are reported to be toxic to algae (Hussaini 1996, Gamila 2004) but their toxicity to other components of aquatic ecosystems is poorly understood.

In the present communication, we are reporting acute toxicity of AlF_3 on a battery of test organisms along with its chronic toxicity to biotic components in an artificial aquatic ecosystem (microcosm) raised in the greenhouse.

MATERIALS AND METHODS

Preparation of stock solution: The test concentrations of aluminium fluoride were prepared separately in potable groundwater (pH = 7.1, hardness = 206 mg/L as $CaCO_3$, chlo-

rides = 30 mg/L, F = 0.9 mg/L) for fish assay, in the lake water (boiled and cooled; pH = 7.6, hardness = 250 mg/L as $CaCO_3$, chlorides = 200 mg/L, F = 0.5 mg/L) for Daphnia assay and in 20 % Hoagland solution made in millipore water for duckweed assay.

Aluminium fluoride is sparingly soluble in water and aluminium ions formed in the soluble state were quantified in all test concentrations of duckweed, Daphnia and fish assays using eriochrome cyanine R method (APHA 1989).

Acute toxicity: Acute toxicity of aluminium fluoride on duckweed (*Lemna aequinoctialis* L.), Daphnia (*Daphnia similis* L.) and fish (*Gambusia affinis* Baird and Gerard) were examined as per methods detailed elsewhere (Sharma et al. 2009). After 96 h of exposure, autopsy of surviving fish was done to perform erythrocyte count and blood smear preparation, according to Lee et al. (1999). Almost 200 erythrocytes in 20 microscopic fields ($10 \times 100_{xs}$) were observed to quantify abnormality in their shape (poikilocytosis) and size (anisocytosis) in a test concentration. LC_{50} and EC_{50} values were calculated using BASICA Software version 1.13.

Chronic toxicity: During winter (November 2009-March 2010), chronic toxicity (at sub-lethal concentration = 35.4 ppm) of aluminium fluoride was examined on flora (algae and aquatic macrophyte) and fauna (zooplankton, snail and fish) in the microcosms developed in 15L plastic buckets

buried 2/3rd in the earthen floor of greenhouse. Each bucket had one outlet, 5 cm below its top while its floor was laid with a 5 cm thick layer of coarse river sand. These were carefully filled with tank water, causing minimum disturbance to the sandy layer. After settlement of suspended particles in the bucket, floating impurities were carefully removed with a sieve. Thereafter, 10-15 cm long, healthy 10 branched shoots of *Ceratophyllum demersum* L. and about 100 *Lemna* plants were added in each bucket. Every morning 10 L of the tank water was introduced gradually in the bucket through a plastic pipe placed just above the sediment. It is important to mention here that various components of aquatic ecosystem have almost naturalized in about 35 years in the tank from where the water has been taken.

On seventh day, 20 mature healthy snails (*Lymnea lueteola* L.) and 35 fish (*Gambusia affinis*) of uniform size (length = 28 ± 1mm; width = 4 ± 1 mm) were transferred gently to each bucket. A plastic mosquito net was tied at the outlet of each bucket to prevent fish loss. In order to meet standard fish diet requirement, 500 mg dried Daphnia powder (Tetrason fish feed) was added daily in each microcosm.

After one month, six microcosms were divided into two groups viz., control and aluminium fluoride treatment. Twice in a week, water in control set was replaced with tank water (10L) while with aluminium fluoride (35.4 ppm) suspension prepared fresh in the tank water for each replicate. Dead fish, if any, were removed regularly.

After 30 and 60 days of exposure, fish were picked up from control and treatment sets and analysed for protein (Lowry et al. 1951), and alkaline and acid phosphatase (Sadasivam & Manickam 1996). Their RBCs were counted and blood smears were prepared for studying morphological abnormalities, as mentioned earlier. Snails (*Lymnea*), counted on day-60, were freed from their shell, and enzymes and protein content in their body and foot were estimated separately. During experiments, animals were maintained as per the guidelines of the Institutional Ethical Committee in the Zoology Department, University of Rajasthan, Jaipur.

Ceratophyllum leaves dried on the blotter were analysed for enzymes and protein after 60 days exposure (Lowry et al. 1951, Sadasivam & Manickam 1996). Shoots were cut in to 5 cm long pieces and those with and without shoot apex are referred to as apical and intercalary shoots respectively hereafter in the text. These were dried on blotter and chlorophyll content in their leaves was estimated, as described elsewhere (Sharma 1985). Apical and intercalary shoots (10 each) were also dried in a hot air oven to constant weight and weighed.

For studying periphyton community, six microscopic glass slides (26 × 76 mm) were hanged just below the water surface in both control and treated microcosms. Three slides each were removed after 7 and 14 days of exposure and their periphyton were scrapped carefully and dispersed in 15mL distilled water containing a drop of Lugol's solution (APHA 1989). Periphyton identified using standard monographs (Pentecost 1984, Tonapi 1980, Battish 1992) were counted using a haemocytometer (algae) and Sedgwick-Rafter cell (zooplankton). Periphyton attached over *Ceratophyllum* leaves were observed under microscope and species present were noted separately in each of 25 observations to calculate their percentage occurrence. The water quality in the microcosms was analysed during the study period by standard methods (APHA 1989).

Statistical analysis: All data presented are mean values of three replicates in each treatment. Student's *t* test was calculated using Systat Version 5.

RESULTS

Acute toxicity of AlF$_3$

Duckweed assay: *Lemna* plants growing in the control sets were bright green having paired fronds. AlF$_3$ exposed plants showed dose dependent etiolation of fronds initiated early (2nd day) at higher concentrations (> 500 ppm) and noted at all test concentrations on 10th day of exposure. Besides, paired fronds were broken into singlet within 96 h of exposure at higher concentrations (3000ppm).

EC$_{50}$ values of AlF$_3$ for chlorophyll content (358.7-780.5 ppm) were higher than that for frond number (285.7-341.5 ppm) suggesting vegetative reproduction in *Lemna* was impaired maximum (Table 1). Similar trend was observed when EC$_{50}$ values were calculated in terms of aluminium concentration (Table 2).

Daphnia assay: A fine milky white turbidity decreased daphniae visibility in AlF$_3$ treatments. It was also deposited on their body. *Daphnia* mortality found nil in control sets was dose dependent in AlF$_3$ treatments. EC$_{50}$ value of AlF$_3$ for *Daphnia* was 108.1 ppm (Table 3).

Fish assay: A fine white precipitate was deposited over the entire fish body, especially on their gill lamellae. The fish moved freely as in control at lower concentrations but their movements declined at higher concentrations (325 ppm-375 ppm) and gills were haemorrhaged. The movements of dying fish were jerky and rolling. When dead, they had opened mouth and flared operculum attributed to asphyxia.

Fish mortality found nil in control and at lower concentrations of AlF$_3$ i.e., < 175 ppm was dose dependent (20-70%) at higher concentrations (225 ppm-375 ppm). LC$_{50}$ value of AlF$_3$ was 354.0 ppm (Table 4).

Aluminium fluoride was found cytotoxic decreasing

RBC counts in the treated fish. EC_{50} value of AlF_3 (307.9 ppm) for RBC counts (concentration at which counts decreased 50%) was lower than that its LC_{50} value (354.0 ppm) suggesting that AlF_3 caused much physiological distress in fish (Table 4). The calculated EC/LC_{50} values in term of aluminium were very low for the test organisms (Daphnia: EC_{50} = 0.23 ppm, fish: LC_{50} = 2.32 ppm).

Morphologically abnormal RBCs (poikilocytosis) were noted both in control and AlF_3 treated fish but their percentage was found higher (13.3-27.8 %) in the latter in comparison to former (5 %) (Fig.1). Most of the abnormal RBCs found in control and AlF_3 treatments were beak shaped and other abnormal morphotypes mostly found in the treatments were spherical, kidney, beaked, triangular, quadrilateral, pentagonal, dumble and tear drop. About 1% RBCs in AlF_3 treatments also had both vacuolization and membrane damage. RBC size also decreased (4 %) in the treated fish (Fig.2).

Chronic Toxicity of AlF_3

Physicochemical characteristics of water: The values of temperature (10-15°C), pH (8.0-8.8) and EC (0.3 mmho/cm) were almost similar in control and AlF_3 exposed microcosms. The dissolved oxygen content was minimum in the morning (Control = 10.08 ± 1.5mg/L, Treatment = 5.85 ± 0.5 mg/L), maximum in the noon (Control = 17.39 ±1.2 mg/L, Treatment = 17.23 ± 1.5mg/L) and moderate in the evening (Control = 14.95 ±0.8 mg/L, Treatment = 16.26 ±1.2mg/L). Its low values in the morning suggest increased respiratory rates of the biotic community, more particularly in the treated microcosms. The values (mean) of total hardness, calcium hardness and chloride content decreased in treatment (TH = 148 mg/L, Ca = 29.6 mg/L, Cl = 46 mg/L) in comparison to control (TH = 176 mg/L, Ca = 35.3 mg/L, Cl = 54 mg/L).

Ceratophyllum: *Ceratophyllum* shoots were healthy and bright green in control sets while showing toxicity symptoms such as etiolation and shedding of leaves in AlF_3 treatments. Shoots were also highly fragile and broke on holding them.

In comparison to control, dry weight and chlorophyll content of shoots decreased in AlF_3 treatment, more particularly of apical shoots, suggesting reduction in the shoot growth (Figs. 3, 4). The exposure also affected tissue biochemistry decreasing protein (53 %), acid phosphatase (56 %) and alkaline phosphatase (14 %) content in the leaves (Fig. 5).

Periphyton: a. Algae: Taxa present in the control sets were; *Coconeis, Cyclotella, Navicula, Tabellaria* (Bacillariophyceae), *Cosmarium, Desmococcus, Oocystis, Oedogonium, Scenedesmus* (Chlorophyceae), and

Microcystis (Cynophyceae). *Oocystis* and *Tabellaria,* found absent in AlF_3 treatment, are considered to be the sensitive taxa. Compared with control, algal counts also decreased significantly (47-54%), being moderate in members of Chlorophyceae (38-41%) but in higher range (61-68 %) for Bacillariophyceae (Fig. 6). *Microcystis* (Cyanophyceae) was, however, found tolerant to AlF_3 since its population increased to almost two folds on day-30. *Navicula*, *Coconeis*, *Cyclotella* and *Scenedesmus* were the sensitive taxa having significant reduction in their population (44-78 %) in AlF_3 treatment.

The composition of algal species associated with *Ceratophyllum* leaves was similar in control and AlF_3 treatment, and these were *Coconeis*, *Desmococcus*, *Navicula*, *Oedogonium, Microcystis* and *Scenedesmus*. The tested chemical was found toxic to only members of Chlorophyceae decreasing their percentage occurrence (5-40%) in comparison to control (20-100%). The percentage occurrence of members of Bacillariophyceae (Control = 70-95%, AlF_3 = 85-95%) and Cyanophyceae (Control and AlF_3 = 20 %) however, differed little.

b. Zooplankton: The species composition in AlF_3 treatment was similar to control and taxa recorded were; *Actinophyra, Coleps, Holophyra, Vorticella* (Protozoa), *Chaetonotus* (Gastrotricha), *Brachionus, Collurella, Lepadella, Lecane, Monostyla, Philodina, Polyarthra, Testudinella* (Rotifera), *Cyclops* and *Nauplius* larvae (Arthropoda).

In contrast to algae, tested chemical had mild toxic effect on the zooplankton population (Fig. 7). Among zooplankton, *Coleps* was the dominant taxon throughout the study period in both control and treatment contributing to almost 95% to the total population. Its counts were higher in the control on day-30 (about 30 %), but in the treatment on day-60 (almost four-folds). This explains why zooplankton counts in control were higher than treatment on day-30 but in the latter on day-60 (Fig. 7). The possible reason for higher *Coleps* counts in the treatment on day-60 may be reduction in predatory pressure of fish on account of toxic effect of the tested chemical. *Vorticella* was the other zooplankton found sensitive to the tested chemical in the first half of the study (day-30) having reduction in counts to more than 90%.

The zooplankton community associated with *Ceratophyllum* leaves was similar to control in AlF_3 treatment and taxa noted were; *Coleps, Collotheca, Monostyla, Vorticella*, *Stentor, Philodina, Polyarthra* and *Testudinella*. Their percentage occurrence also differed little in control (10-75%) and AlF_3 treatment (10-100%).

Snail: A fine white deposit was observed on the shell and

Table 1: EC_{50} values (4th, 7th and 10th day) of AlF_3 for duckweed.

Day	EC_{50} based on	EC_{50} (ppm)	Probit Regression Line	95% Confidence limit Upper	Lower
4th day	Frond number	341.5	Y = -21.11519 + 10.30826X	2259.7	51.6
	Chlorophyll	780.5	Y = 8.405686E-03 + 1.725751X	3040.6	200.3
7th day	Frond number	555.4	Y = -8154001 + 2.1188X	1197.2	257.6
	Chlorophyll	406.0	Y = -5.7388 + 4.124143X	534.7	308.2
10th day	Frond number	285.7	Y = -8.1157 + 5.340355X	419.5	194.5
	Chlorophyll	358.7	Y = 10236312 + 10473178X	622.5	206.7

Table 2: EC_{50} values (4th, 7th and 10th day) of Al based on estimated values in test concentrations of AlF_3 for duckweed.

Day	EC_{50} based on	EC_{50} (ppm)	Probit Regression Line	95% Confidence limit Upper	Lower
4th day	Frond number	3.14	Y = -0.125 + 10.309X	20.78	0.47
	Chlorophyll	7.18	Y = 3.52 + 1.72 X	27.99	1.84
7th day	Frond number	5.11	Y = 3.498 + 2.119X	11.01	2.37
	Chlorophyll	3.74	Y = 2.77 + 3.898X	4.92	2.83
10th day	Frond number	2.63	Y = 2.758 + 5.34X	3.86	1.79
	Chlorophyll	3.30	Y = 4.23 + 1.47X	5.73	1.90

Table 3: EC_{50} values of AlF_3 for *Daphnia*.

EC_{50} (ppm)	Probit Regression Line	95% Confidence limit Upper	Lower
108.06*	Y = 1.78789 + 1.579464X	270.69	43.15
0.23**	Y = 6.02 + 1.58X	0.56	9.08E-02

*Based on test concentrations; **Based on estimated values of Al in test concentrations of AlF_3

Table 4: LC_{50} (mortality) and EC_{50} values (RBC counts) of aluminium fluoride for fish.

Parameters	LC/EC_{50} (ppm)	Probit Regression Line	95% Confidence limit Upper	Lower
Mortality	354.0*	Y = 6.495765 + 4.509876X	657.4	190.6
RBC counts	307.9*	Y = -2.041504 + 2.829644X	341.3	277.8
Mortality	2.32**	Y = 2.89 + 5.77X	3.57	1.50
RBC counts	2.27**	Y = 3.99 + 2.82X	2.52	2.05

*Based on test concentrations; **Based on estimated values of Al in test concentrations of AlF_3

foot of the snails in the aluminium fluoride treatment. Similar to control, snail mortality in AlF_3 treatment was nil but their movements slowed down. The exposure to test chemical, however, had marked effect on tissue biochemistry, as evident by significant alterations in values of ACP, ALP and protein content of body and foot (Fig. 8).

Fish: Similar to snail, a fine white deposit was noted on aluminium fluoride exposed fish, more particularly, on their gills. In comparison to control fish, treated fish had low appetite as daphnia food offered to them remained suspended in the microcosms. They also had higher mortality (37%) when compared with control (5 %). As noted earlier, AlF_3

had cytotoxic effects on RBCs causing poikilocytosis and microcytic anaemia (Figs. 9, 10, 11).

The alteration in fish biochemistry included reduction in acid phosphatase content during the study period (Fig. 12). Alkaline phosphatase and protein content also decreasing on day-30 followed an opposite trend on day-60 (Fig. 12).

DISCUSSION

Present study has revealed mild toxicity of aluminium fluoride to a battery of test organisms. The comparison of LC_{50}/EC_{50} values revealed *Daphnia* to be the most sensitive
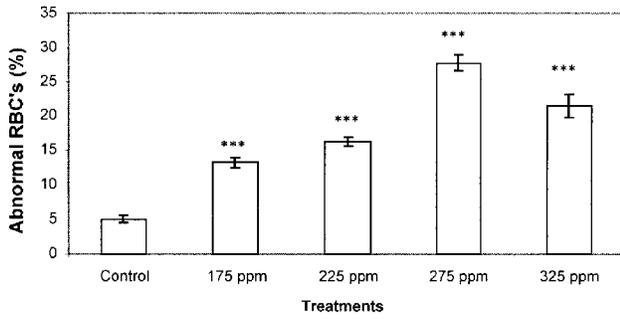
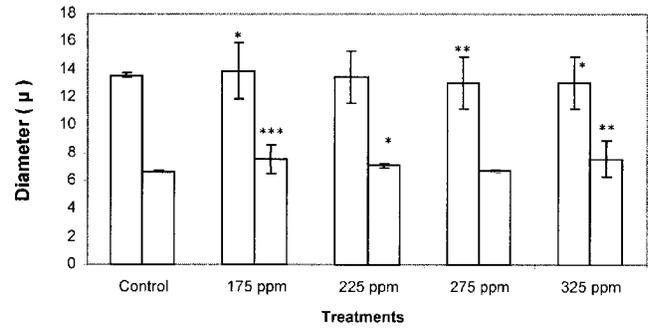Fig. 1: Percentage of abnormal RBC's in fish in control and AIF₃ treatments.

Fig. 2: Diameter of RBC and nucleus (μ) in control and aluminum fluoride exposed fish. (Significant at *5% **1% and ***0.1% probability)
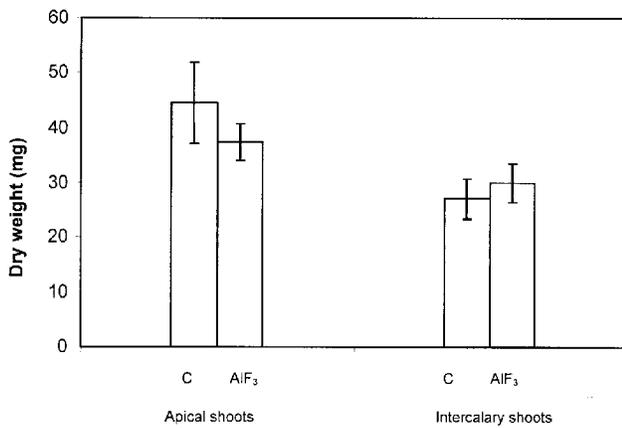

Fig. 3: Dry weight (mg) of apical and intercalary shoots of *Ceratophyllum* growing in the controls and aluminum fluoride treatments.
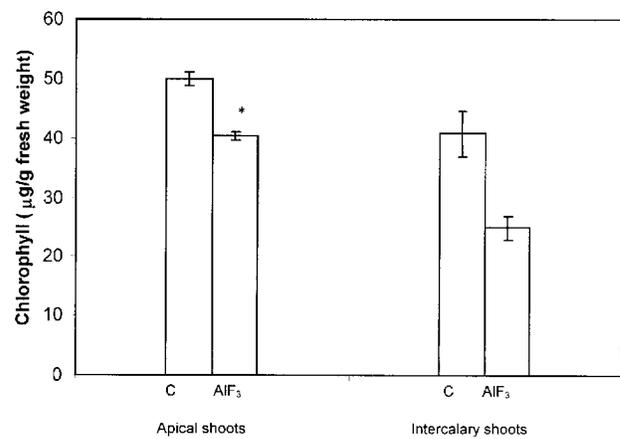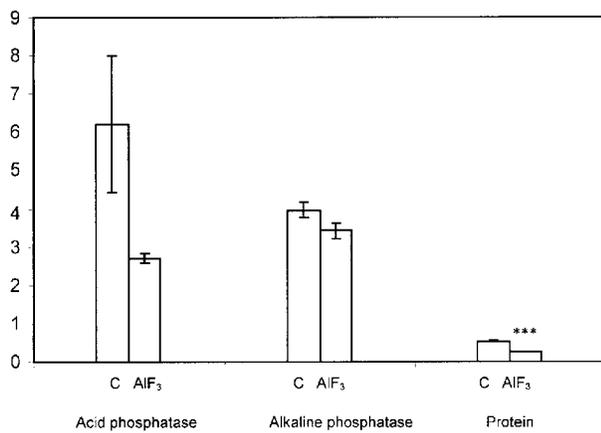

Fig. 4: Chlorophyll content (μg/g fresh weight) in apical andintercalary shoots of *Ceratophyllum* growing in the controls and aluminum fluoride treatments (Significant at *5% probability).

Fig. 5: Protein (mg/g), alkaline and acid phosphatase (μmoles/mg) content in the leaves of *Ceratophyllum* shoots growing in the controls and aluminum fluoride treatments (Significant at ***0.1% probability).
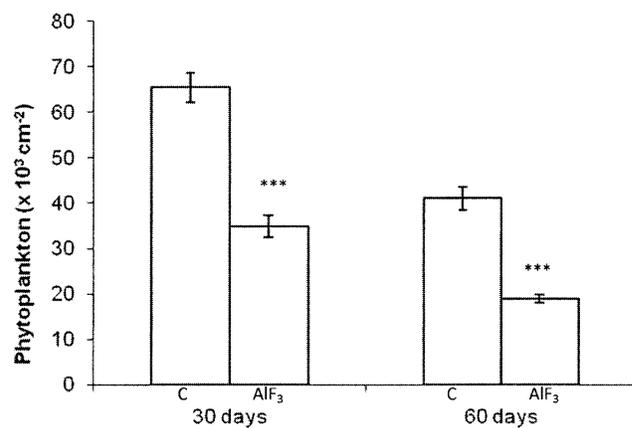

Fig. 6: Periphyton (algae) counts in the control and AIF₃ exposed microcosms by slide study (*** significant at 0.1% probability).
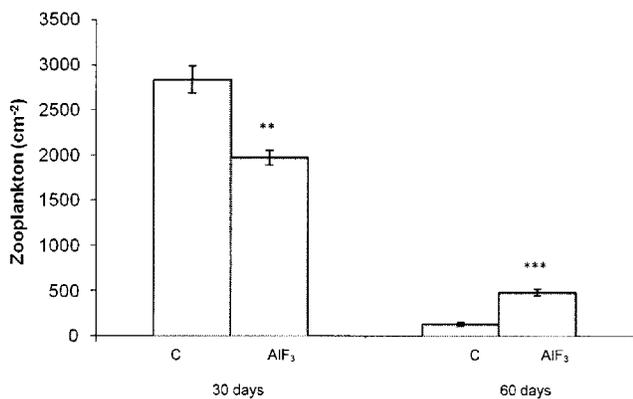
Fig. 7: Periphyton (zooplankton) counts in the control and AIF₃ exposed microcosms by slide study (significant at ** 0.1% *** 0.1% probability).
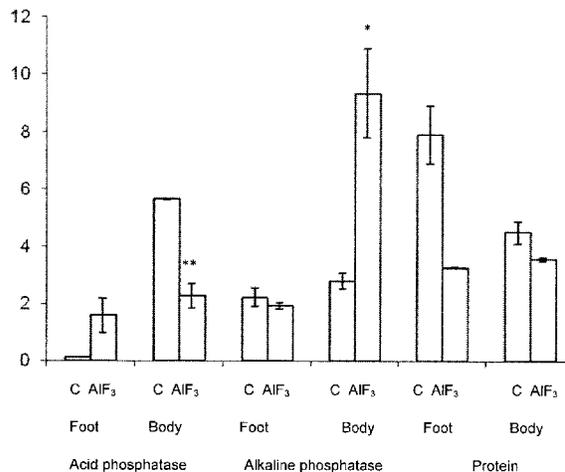

Fig. 8: Acid and alkaline phosphatase (μmoles/mg) and protein (mg/g) content of Lymnea reared in the controls and aluminum fluoride treatment (Day 60).
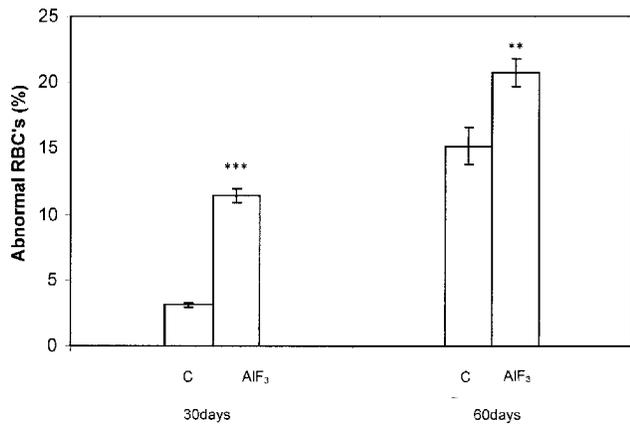

Fig. 9: RBC counts ($\times 10^4$ mm^{-3}) in the controls and aluminum fluoride treatment (significant at **1% probability).
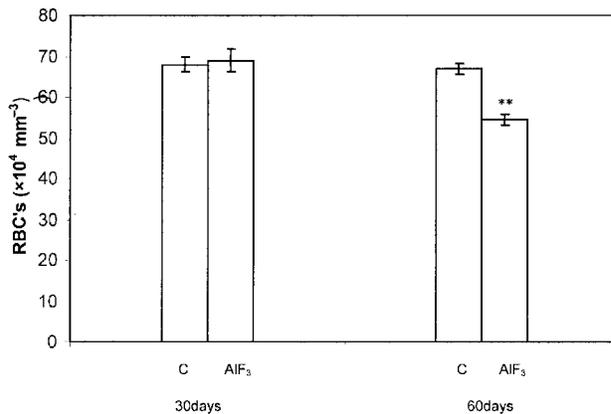

Fig. 10: Percentage of normal and abnormal RBCs (Poikilocytosis) in control and aluminum fluoride exposed fish (significant at **1% and ***0.1% rpobability).
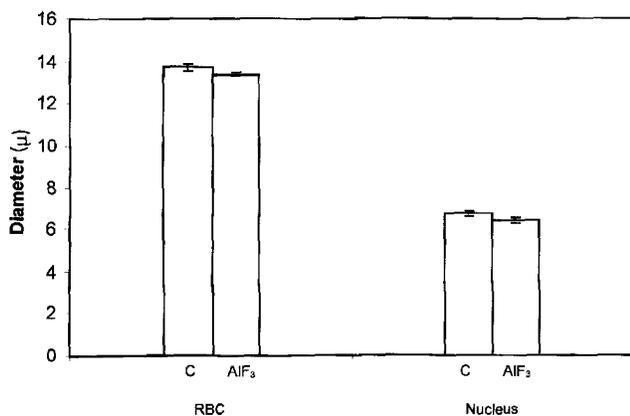

Fig. 11: Diameter of RBC and nucleus (μ) in control and aluminum fluoride exposed fish.
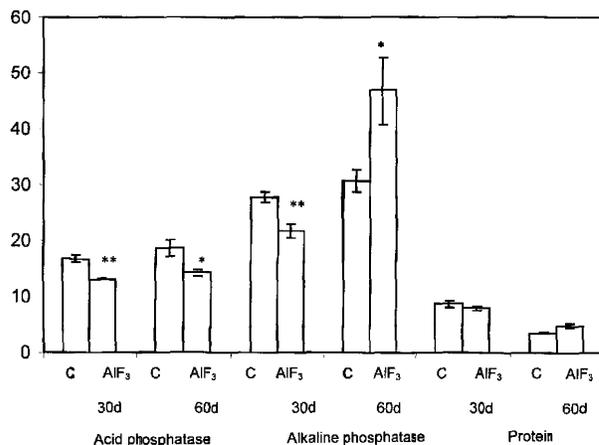

Fig. 12: Protein (mg/g), alkaline and acid phosphatase content (μmole/mg) in control and aluminum fluoride exposed fish (Significant at *5%, **1% probability).

test organism followed by fish and *Lemna* (Table 1, 2, 3, 4). The mild toxicity of AlF_3 may be ascribed to its poor solubility in water. When aluminium fluoride toxicity in term of Al^{+3} in the test concentrations is compared with values reported in literature, *Gambusia* fish in the present study were relatively more tolerant to *Tilapia zillii* ($LC_{50} = 125$ μg, Alwan et al. 2009) but sensitive in comparison to *Brachydanio rerio* ($LC_{50} = 56.92$ ppm, Anandhan & Hemlatha 2009).

The chronic exposure of aluminium fluoride adversely affected primary producers in the microcosm (Figs. 3, 4, 6). Other workers also made similar findings. AlF_3 has been reported to inhibit Mg^{2+} and Ca^{2+}-ATPase activities in *Nostoc linckia* and *Chlorella vulgaris* (Husaini et al. 1996) and suppresses photosystems (PS-I and PS-II) in *Nostoc linkia* (Rai et al. 1996) that finally may reduce algal growth. These workers found $AlCl_3$ to be more toxic than AlF_3 that increased further in combination with NaF with increasing acidity. Stevens et al. (1997) made interesting findings for land plants reporting aluminium to be most toxic, AlF^{2+} toxic to lesser extent, and AlF_3, AlF_4^- and F^- are the least toxic. Mo et al. (1988) examined toxicity of Al and Cu in relation to pH on duckweed. They reported that the Mg^{2+} in chlorophyll was replaced by the Cu^{2+} or Al^{3+} which may lead the chlorophyll to lose its normal activity and kill the duckweed. Based on these findings, it may be concluded that aluminium fluoride is toxic to primary producers in the aquatic ecosystems.

AlF_3 exposure induced stress in *Ceratophyllum* shoots that decreased its protein, acid phosphatase and alkaline phosphatase content (Fig. 5). The exposure to heavy metals also decreased acid phosphatase activity in the roots of *Alyssum* species and cucumber (*Cucumis sativus* L.) seedlings (Gabbrielli et al. 1999, Tabaldi et al. 2007). Similar trend was noted in alkaline phosphatase activity of *Scenedesmus* when aluminium and copper were added to chemically defined media (Rueter et al. 1987). Such reduction in the availability of biomolecules may disturb plant metabolism.

Zooplankton are one of the most important biotic components influencing all the functional aspects of an aquatic ecosystem, such as food chains, food webs, energy flow and cycling of matter (Park & Shin 2007). They are well suited tool for understanding water pollution status (Contreras et al. 2009). aluminium fluoride toxicity to zooplankton seems to be species specific, found toxic to *Daphnia* (during acute toxicity), *Coleps* and *Vorticella* during first half of chronic exposure. The exposure to aluminium has been reported to impair ion regulation and respiratory efficiency in *Daphnia* and taxa belonging to class Ephemeroptera, Plecoptera and Cladocera (Havas & Likens 1985, Sparling & Lowe 1996, Soucek 2006). Though we have no data to support these find-

ings but it is likely that aluminium fluoride exposure may also have similar effects on zooplankton.

Snails feeding on ooze and dead animal matter are considered as useful indicator species for biological assessment of water quality (Nesemann & Sharma 2005). Though *Lymnea* had no mortality in the present study but its movements slowed down in AlF_3 treatment. Campbell et al. (2000) also reported depression of behavioural activity in snails (*Lymnaea stagnalis*) exposed (7 days) to aluminium nitrate, aluminium lactate and aluminium maltol.

Fish mortality was recorded during both acute and chronic exposures. Beside toxic nature of the chemical, fish mortality may also be ascribed to asphyxia caused by its deposition on their gills. Moss & Hathway (1964) reported permeability of erythrocyte membrane to the pollutants, which may reduce life span and production of erythrocytes due to damage of erythrogenic tissue causing deficiency of all or some cellular elements in peripheral blood (McLeay 1973). AlF_3 is also known for inducing morphological abnormalities in RBC caused by change in structure and functions of cell membranes (Suwalsky et al. 2004, Hernandez et al. 2008). These findings explain reduction in RBC counts and their morphological abnormalities in fish during acute and chronic exposure (Figs. 1, 9, 10). Such haematological alterations may adversely affect oxygen carrying capacity of the blood and thereby overall metabolism.

Pollutants exposure also affects biochemical parameters in animals. The reduction in protein content in both snail and fish may be ascribed to proteolysis and delay in protein synthesis, as reported by Kumar et al. (2007) in the freshwater male catfish (*Clarias batrachus*) exposed to lower and higher F concentration (NaF: 35mg F ion/L and 70 mg F ion/L). The loss in fish appetite as explained earlier and possible use of body reserve such as protein to meet respiratory demand may also explain reduction in protein content.

Alkaline phosphatase and acid phosphatase susceptibility to toxic challenge is well established in vertebrates (De Boeck et al. 2001, David et al. 2003) and to some extent in invertebrates (Satyaparameshwar et al. 2006, Lodhi et al. 2006) and so their profiling is a commonly used diagnostic tool to quantify stress imposed by environmental pollutants in living organisms (Cheng 1983, Santhakumar et al. 2000). These enzyme activities were altered in snail and fish exposed to aluminium fluoride suggesting them to be under stress.

Present study has thus revealed that biotic components of aquatic ecosystem were under stress. The low oxygen content in the treated microcosms during morning ascribed

to high respiration rate of the biotic community support this view point.

ACKNOWLEDGEMENTS

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