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Original Research Paper

Toxic Effects of Aluminium and Fluoride on Planktonic Community of the Microcosms

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ABSTRACT

Aluminium and fluoride were found toxic to phytoplankton, periphyton and zooplankton in the microcosms raised in the greenhouse. The toxicity of aluminium was relatively higher than fluoride, more particularly, during winter season. There were significant reduction in counts of phytoplankton (49-80%), periphyton (algae = 40-68%, zooplankton = 35-75%) and zooplankton (5-77%) in the treatments in comparison to controls. As a result, Reciprocal Simpson index, Equitability index and Shannon- Weiner index decreased. Such changes will affect energy flow in the microcosms.

INTRODUCTION

Aluminium finds its way in the environment through coal strip mining activities, water treatment facilities using aluminium sulphate (alum) as a coagulant for suspended solid particles removal, industrial wastes and acid rainfall (Alwan et al. 2009), whereas production of steel, aluminium, ceramics and phosphate fertilizer including coal combustion contribute to fluoride pollution (Smith & Hodge 1979, Hillier 2000).

Aluminium and fluoride are toxic to algae such as *Chlorella pyrenoidosa* (Parent & Campbell 1994), *Chlorella fusca* and *Chaetomorpha brachygona* (Wong et al. 1994), *Chlorella vulgaris* (Rai et al. 1998), *Dunaliellla tertiolecta* (Sacan et al. 2007), *Chlamydomonas gigantean* (Quiroz-Vázquez et al. 2010), *Anabaena khannae* and *Chlorococcum humicola* (Bhatnagar & Bhatnagar 2004), *Synechococcus leopoliensis* (Nichol et al. 1987); and zooplankton such as *Skistodiaptomus oregonensis* (Havens 1993), *Daphnia magna* (Hickey 1993, Dave 1984) *Lecane quadridentata* (Guzman et al. 2010) and *Daphnia carinata, Simocephalus vetulus, Eriodaphnia dubia* and *Ceriodaphnia* cf. *pulchella* (Hickey 1989).

Planktons are effective bioindicator of pollution in the aquatic environments (Chandra & Kulshreshtha 2004) and are well suited tool for understanding their pollution status (Contreras et al. 2009). The exposure of phytoplankton to pollutants may have various influences on the aquatic ecosystems, either by initiating a chain of bioaccumulation (Rai et al. 1981, Jensen et al. 1982 a, b) or by inhibiting the flow of energy into food webs. Since phytoplankton are primary producer of organic compounds in the aquatic systems and 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), there is a need to determine the effects of fluoride and aluminium on phyto and zooplankton.

In the present study, we report aluminium and fluoride toxicity on plankton community in artificial microcosms raised in the greenhouse.

MATERIALS AND METHODS

Dissolving weighed amount of Analar grade aluminium sulphate ($Al_2(SO_4)_3.16H_2O$) and sodium fluoride (NaF) in distilled water, 10000ppm stock solutions were prepared. Microcosms (24) developed in 15L plastic buckets, as described elsewhere (Sharma et al. 2003), were divided into four groups of 6 microcosms each, based on feed provided to fish in addition to their natural feed. *Daphnia* powder (500mg/microcosm/day, Tetrason Real Gold) was added to all 24 microcosms. In addition, *Spirulina*, tamarind pulp and *Spirulina* + tamarind pulp were fed to fish in microcosms of group 2, 3, 4, as described elsewhere (Sharma et al. 2012). Based on extra feed, these four groups of microcosms are hereafter, referred to as Daphnia (D), Daphnia + Spirulina (SD), Daphnia + Tamarind (TD) and Daphnia + Spirulina + Tamarind (STD) microcosms in the text.

After one month, six microcosms of a group were segregated into three sub-groups *viz*., control, aluminium sulphate and sodium fluoride, each having 2 microcosms. Every alternate day, water in control sets was replaced with tank water (10L) wherein producers and consumers have almost naturalized in more than 30 years while with aluminium sulphate (3 ppm) and sodium fluoride (10 ppm) prepared by diluting their stock solutions (1000 ppm) with tank water for 60 days in the treated microcosms.

About 500 mL of water sample collected from each of the microcosm was centrifuged at 3000 rpm for 20 minute. The concentrate was finally made up to a known volume by distilled water and later transferred in polyethylene sample bottle for storage adding 1mL of Lugol's solution per 100mL of solution.

Using standard monographs, both phytoplankton (only in D microcosms) and zooplankton were identified (Smith 1950, Pentecost 1984, Tonapi 1980, Battish 1992). Phytoplankton (only in D microcosms) and zooplankton were counted using Haemocytometer and Sedgwick-Rafter Cell respectively (APHA 1989).

Periphytons were also studied by hanging six microscopic glass slides (26 × 76 mm) just below water surface in each microcosm. Three slides were removed after 7 and 14 days of exposure and their periphyton were removed carefully with a razor blade. Scrapings were dispersed in 15mL of distilled water containing 1-2 drops Lugol's solution and periphyton were counted (APHA 1989).

RESULTS

Effects on Phytoplankton

Summer season: Fluoride (F^-) exposure altered species composition replacing *Chlorococcum* present in control with *Oscillatoria*. *Chlorococcum* and *Navicula* present in the control were however, absent in Al^{3+} treatment. Thus, overall species richness found similar to control in F^- treatment decreased in Al^{3+} treatment (Table 1).

Phytoplankton counts, however, decreased (49-51%) because of significant reduction in *Cocconeis, Desmococcus* and *Navicula* counts (38-91%) in the treatments (Fig. 1). In contrast, members of Cyanophyceae were either having higher counts (*Microcystis*) or exclusively present (*Oscillatoria*) in the treatments.

Such perturbations in species richness and their counts affected diversity indices. The values of Reciprocal Simpson index, Equitability index and Shannon-Weiner index were higher than control in F^- treatment but their values decreased in Al^{3+} treatment. In comparison to control, F^- exposure increased evenness, dominance and biodiversity of phytoplankton while these indices decreased in Al treatment (Table 1).

Winter season: The exposure to test chemicals altered species composition. Taxa such as *Ankistrodesmus, Oocystis, Micrasterias* and *Microcyctis* were absent in treatments whereas *Acanthes* and *Pinnularia* were present only in the treatments. As a result, species richness (7-9) decreased, more particularly in F^- treatment, when compared with control (10 species, Table 1).

Compared with control, there was reduction in phytoplankton counts (62-80%) in treatments, more particularly in Al^{3+} treatment because of significant reduction (40-96%) in the counts of *Cocconeis, Chlamydomonas, Chlorella, Navicula, Scenedesmus* and *Microcystis* (Fig. 1).

The perturbations in species richness and their counts decreased values of Reciprocal Simpson index, Equitability index and Shannon-Weiner index because of reduction in evenness, dominance and biodiversity of phytoplankton (Table 1).

Effects on Zooplankton

Summer season: The diet supplements had favourable effects increasing species richness and counts of zooplankton (Table 2). Species richness of zooplankton was found almost similar to controls in F^- and Al^{3+} treatments but their counts decreased (5-40%) in Al^{3+} treatments, with the exception of SD microcosm (Fig. 2).

Compared with controls, Reciprocal Simpson index, Equitability and Shannon-Weiner indices increased in the treatments of D microcosms, but decreased in the diet supplement microcosms (Table 2). The test chemical exposures thus increased evenness, dominance and biodiversity of zooplankton in D microcosms but these decreased in others. Such alterations in values of indices were on account of perturbations in species richness and counts of zooplankton populations, more particularly in SD and STD microcosms. There was a large scale build up of *Polyarthra* and *Coleps* in the microcosms while populations of other species declined.

Winter season: Species richness and counts of zooplankton were higher in the diet supplement microcosms compared with D microcosms (Table 3). Test chemicals having little adverse effects on species richness were, however, found more detrimental to zooplankton population decreasing their counts (22-77%) in all microcosms, more particularly in D and TD microcosms (Fig. 2).

Reciprocal Simpson index, Equitability index and Shannon Weiner index decreased in treatments of D microcosms but their values increased in the treatments of diet supplement microcosms. Test chemicals decreasing evenness, domi-

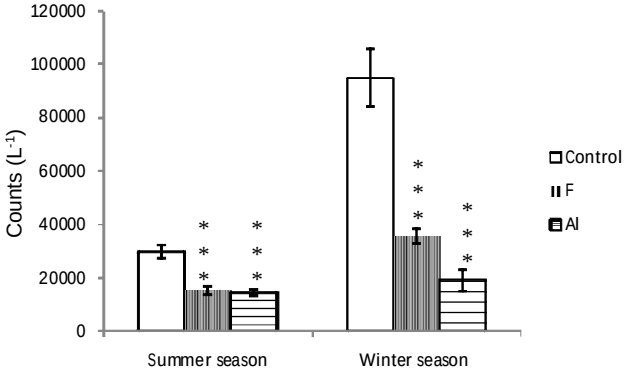

Fig. 1: Phytoplankton counts in the control, fluoride and aluminum exposed microcosms during summer and winter season; ***significant at 0.1 % probability.

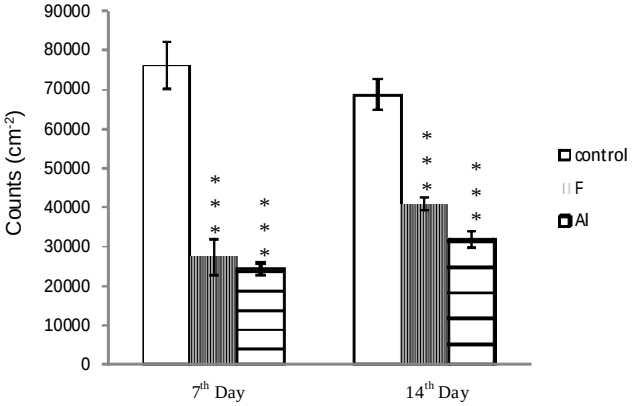

Fig. 3: Periphyton (phytoplankton) counts (7 & 14 days) in the control, fluoride and aluminum exposed microcosms (Slides study) during summer season, *** Significant at 0.1 % probability

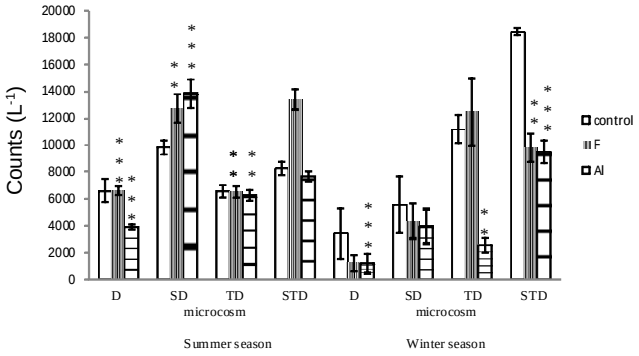

Fig. 2: Zooplankton counts in the control, fluoride and aluminum exposed microcosms during summer and winter season; **significant at 1.0%, and *** 0.1 % probability.

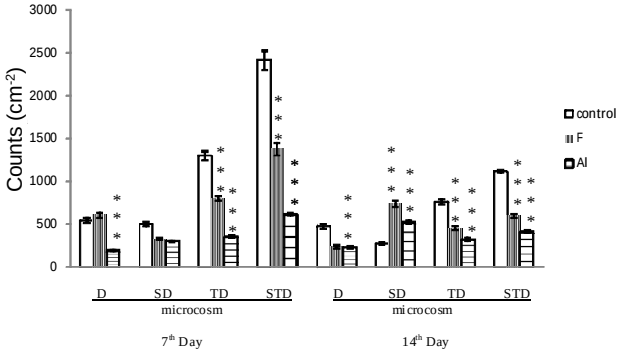

Fig. 4: Periphyton (zooplankton) counts (7 & 14 days) in the control, fluoride and aluminum exposed microcosms (Slides study) during summer season, *** Significant at 0.1 % probability

nance and biodiversity of zooplankton in the D microcosms however, favoured them in the diet supplement microcosms. Such alterations have been ascribed to buildup in population of *Amoeba* cyst, *Euglena, Holophrya, Monostyla* and *Nauplius* larvae in both controls and treatments of diet supplement microcosms.

Effects on Periphyton During Summer

Phytoplankton: Test chemicals exposure decreased species richness (20-75%) and counts (40-68%) of periphyton, more particularly in Al^{3+} treatment, when compared with control. Toxic effects were observed on selective species (Table 4, Fig. 3). There was significant reduction (30-75%) in the counts of *Cocconeis, Desmococcus, Navicula* and *Oscillatoria*, and taxa such as *Pandorina* and *Cosmarium* were even absent.

Reciprocal Simpson index and Shannon-Weiner index decreased in the treatments but Equitability index increased suggesting that exposure to test chemicals increased homogeneity in phytoplankton population but their dominance and biodiversity decreased, more particularly in Al^{3+}, when compared with control.

Zooplankton: F^- and Al^{3+} exposure affected species richness of zooplankton little but their counts decreased significantly (35-75%) in comparison to controls, especially in Al^{3+} treatments (Table 5, 6; Fig. 4). Interestingly, there was a buildup in the population of *Chaetonotus , Coleps, Lepadella* and *Vorticella* in both controls and treatments of the diet supplement microcosms, more particularly in STD microcosm, when compared with D microcosms. Whereas taxa such as *Colotheca*, *Heliozoan*, *Lecane* and *Limnias* were absent in treatments.

Seven days exposure to test chemicals increased values of all the three indices suggesting greater evenness, dominance and biodiversity in the treatments in comparison to controls. However, almost opposite trends were observed

Table 1: Species richness, counts (L^{-1}) and diversity indices of phytoplankton in the controls, fluoride and aluminum exposed microcosms during summer and winter seasons.

Phytoplankton	Summer season			Winter season		
	C	F^-	Al^{+3}	C	F^-	Al^{+3}
Species richness	5	5	3	10	7	9
Counts (L^{-1})	29800	15300	14500	95000	35900	19100
Reciprocal Simpson index (1/D)	1.268	1.383	1.059	1.536	0.7199	0.7259
Shannon- Weiner index (log)	1.649	1.658	1.278	2.142	1.077	1.158
Equitability index (Evenness)	0.7101	0.7139	0.8065	0.6448	0.3836	0.3654

Table 2: Zooplankton counts in the controls, fluoride and aluminum exposed microcosms during summer season.

Zooplankton	D microcosm			SD microcosm			TD microcosm			STD microcosm		
	C	F^-	Al^{+3}	C	F^-	Al^{+3}	C	F^-	Al^{+3}	C	F^-	Al^{+3}
Species richness	9	9	9	12	10	11	8	9	10	12	13	10
Counts (L^{-1})	6590	6620	3950	9830	12730	13830	6560	6520	6250	8280	13400	7660
Reciprocal Simpson index(1/D)	0.909	0.978	1.054	2.296	0.847	1.319	1.266	1.523	0.801	1.132	1.086	0.772
Shannon- Weiner index (log)	1.54	1.676	1.795	2.656	1.455	1.847	1.982	2.122	1.361	1.898	1.864	1.144
Equitability index (Evenness)	0.4859	0.528	0.566	0.740	0.438	0.534	0.660	0.669	0.409	0.529	0.503	0.344

Table 3: Species richness, counts (L^{-1}) and diversity indices of zooplankton in the controls, fluoride and aluminum exposed microcosms during winter season.

Zooplankton	D microcosm			SD microcosm			TD microcosm			STD microcosm		
	C	F^-	Al^{+3}	C	F^-	Al^{+3}	C	F^-	Al^{+3}	C	F^-	Al^{+3}
Species richness	8	5	8	7	5	7	10	10	10	13	13	11
Counts (cm^{-2})	3430	1220	1220	5570	4350	3970	11190	12480	2590	18420	9800	9500
Reciprocal Simpson index(1/D)	1.505	1.267	0.9387	1.173	1.603	1.427	1.573	2.487	2.719	2.526	3.626	3.281
Shannon- Weiner index (log)	1.89	1.635	1.489	1.457	1.926	1.795	2.072	2.527	2.737	2.609	3.128	2.974
Equitability index (Evenness)	0.6299	0.7041	0.4963	0.5191	0.8294	0.6395	0.6236	0.7606	0.8241	0.705	0.8454	0.8596

Table 4. Species richness, counts (cm^{-2}) and diversity indices of periphyton (phytoplankton) at day-7 and 14 in the control, fluoride and aluminum exposed microcosms during summer season.

Phytoplankton	Day- 7			Day -14		
	C	F^-	Al^{+3}	C	F^-	Al^{+3}
Species richness	8	4	2	5	4	3
Counts (cm^{-2})	76240	27500	24400	68600	40900	32000
Reciprocal Simpson index (1/D)	2.004	1.249	0.987	1.57	1.432	1.168
Shannon- Weiner index (log)	2.26	1.559	0.9905	1.814	1.649	1.395
Equitability index (Evenness)	0.7535	0.7797	0.9905	0.7814	0.8244	0.8799

after 14 days exposure. These findings suggest increase in test chemicals toxicity with exposure period.

DISCUSSION

Fluoride and aluminium had higher toxicity to phytoplankton and periphytic algae compared with zooplankton (Figs. 1-4). *Ankistrodesmus, Chlorococcum, Cocconeis, Cosmarium, Desmococcus, Oocystis, Micrasterias, Navicula* and *Pandorina* were the most sensitive taxa to test chemicals while *Acanthes, Pinnularia, Microcystis* and *Oscillatoria* were the tolerant one. Aluminium was found to be relatively more toxic to phytoplankton. Toxicity varied seasonally and was found higher during winter (Figs. 1, 2).

Aluminium was found to be toxic to zooplankton in both summer and winter seasons but fluoride toxicity was observed only during winter. The percentage reduction in

Table 5: Species richness, counts (cm^{-2}) and diversity indices of periphyton (zooplankton) at Day-7 in the control, fluoride and aluminum exposed microcosms during summer season.

Zooplankton	D microcosm			SD microcosm			TD microcosm			STD microcosm		
	C	F^-	Al^{+3}	C	F^-	Al^{+3}	C	F^-	Al^{+3}	C	F^-	Al^{+3}
Species richness	10	10	6	9	7	8	11	9	10	10	12	9
Counts (cm^{-2})	551	606	198	501	331	302	1301	803	362	2413	1380	617
Reciprocal Simpson index(1/D)	1.868	2.115	2.572	1.757	1.807	2.294	2.592	2.27	2.702	1.263	1.854	1.282
Shannon- Weiner index (log)	2.416	2.432	2.447	2.152	2.166	2.475	2.699	2.471	2.669	1.686	2.208	2.029
Equitability index (Evenness)	0.7271	0.7321	0.9468	0.6787	0.7716	0.8252	0.7803	0.7794	0.8035	0.5075	0.6158	0.6402

Table 6: Species richness, counts (cm^{-2}) and diversity indices of periphyton (zooplankton) at Day-14 in the control, fluoride and aluminum exposed microcosms during summer season.

Zooplankton	D microcosm			SD microcosm			TD microcosm			STD microcosm		
	C	F^-	Al^{+3}	C	F^-	Al^{+3}	C	F^-	Al^{+3}	C	F^-	Al^{+3}
Species richness	10	8	7	7	12	11	12	9	8	8	8	8
Counts (cm^{-2})	477	239	231	277	740	526	765	459	325	1113	598	413
Reciprocal Simpson index(1/D)	2.456	2.352	1.786	1.831	1.344	1.795	3.071	2.059	2.222	1.344	1.388	2.114
Shannon- Weiner index (log)	2.626	2.512	2.26	2.236	2.114	2.463	2.89	2.481	2.432	1.808	1.874	2.464
Equitability index (Evenness)	0.7904	0.8372	0.805	0.7965	0.5896	0.7119	0.8062	0.7828	0.8105	0.6027	0.6246	0.8214

zooplankton counts was also higher during winter season (Figs. 2, 4). The higher toxicity to phytoplankton and zooplankton suggests synergistic effect of low temperature on toxicity of test chemicals.

Interestingly, there was buildup in zooplankton population in both controls and treatments of microcosms wherein fish were provided diet supplement *Spirulina* and tamarind alone and also in combination. This may be ascribed to reduction in grazing pressure of fish on zooplankton because of their greater preference to diet supplements (Sharma et al. 2012).

The perturbations in plankton populations affected community structure. The values of indices such Equitability index, Reciprocal Simpson index and Shannon-Weiner index often decreased for phytoplankton, periphyton and zooplankton during the study period, suggesting reduction in their evenness, dominance and biodiversity (Tables 1-6).

The reduction in algal counts will adversely affect energy flow in F^- and Al^{3+} treatments. It will also affect food availability to grazers such as zooplankton and fish feeding on planktonic algae such as *Chlorococcum*, *Cosmarium*, *Desmococcus*, *Navicula*, *Oocystis* and *Pandorina*, which were recorded during gut content analysis of microcosm fish (Unpublished data). These grazers do not feed on tolerant algae such as *Microcystis* and *Oscillatoria* (also filamentous) because of their mucilaginous coating and toxin content.

Present study revealed higher toxicity of Al^{3+} to phytoplankton, periphyton and zooplankton in the microcosms in comparison to fluoride. In an earlier study, we have reported them toxic to snail and fish in the microcosms (Sharma et al. 2012). In view of these findings, we conclude toxic nature of test chemicals in the aquatic ecosystems, more so, of aluminium.

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