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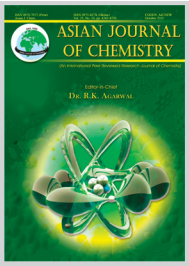

Studies on Aluminium Uptake by the Seeds Under Different Chemical Milieu and Its Effect on Seed Germination and Plant Growth of Bengal Gram (*Cicer arietinum*), Pea (*Pisum sativum*) and Horse Gram (*Dolichos biflorus*)

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Aluminium uptake by the seeds of bengal gram (*Cicer arietinum*), pea (*Pisum sativum*) and horse gram (*Dolicos biflorus*) under different chemical milieu have been studied. Effect of aluminium absorption on the germination of seeds, as well as, the root and shoot length of the plants out of germinated seeds have also been studied. Effect of other cation *viz*., Ca^{2+} and anions *viz*., malate, tartarate and citrate on the aluminium absorption have also been studied. The seeds were also grown in earthen pots in soil cultures, with soils mixed with different aluminium ion concentration. The shoot length, number of leaves as well as nature of roots were observed after a definite time interval. Results revealed an expression of toxicity by the seeds upon exposure to the aluminium ions in solution of different concentrations. The germination was severely affected upon deep as well as shallow soaking of seeds in Al^{3+} solutions. Bengal gram germination was highly affected right from 100 ppm of Al^{3+} only. At 100 ppm of Al^{3+} it showed only 4 % germination, whereas pea and horse gram were relatively less affected by Al^{3+} toxicity. With increasing Al^{3+} concentration, all the three species of seeds increased Al uptake gradually. Comparative results of 24 and 48 h soaking time suggest that kinetically 24 h period seem to be sufficient time to express full toxicity and maximum Al^{3+} uptake. The plants germinated out of intoxicated seeds showed a depressed root and shoot length. Presence of Ca^{2+} or chelating anions like malate, tartarate and citrate could not alleviate aluminium toxicity to any significant extent for any of the seeds. In soil culture the Al toxicity was also expressed, however, to a lesser extent than that in solution intoxication. The morphogenic aspects such as shoot length and number of leaves were severely affected, particularly in case of horse gram, in soil culture.

Key Words: Aluminium toxicity, Aluminium phytotoxicity, Aluminium toxicology, Aluminium ecotoxicology.

INTRODUCTION

Aluminium is the third most abundant element in the earth's crust. It comprises *ca.* 8 % of the outer 16 kms of the crust. It is a highly reactive metal and hence does not occur free in nature. It occurs chiefly as oxides and complex alumino-silicates. Though aluminium is one of the most abundant metals in the biosphere, it does not have any useful biological function. It has been rather proved to be toxic to human body[1-14]. Aluminium has been implicated as a potential neurotoxic factor in different pathological conditions[6,7]. The extensive use of aluminium cookware and food packaging material and use of aluminium salts in food additives and some drugs, provide potential sources of aluminium ingestion.

Though aluminium is well protected from its bioavailability in nature by the formation of stable complex alumino-silicates, nevertheless in case of disbalances in ecosystems, such as acid rains or a decrease in soil pH by industrial effluents and consequent water pollution, there may be leaching of aluminium ions and their consequent entry into the flora and fauna through the aquatic systems. This evidently would lead to onward transmission of aluminium ions in the food chains of the ecosystems and hence create toxicity. Aluminium may enter into the body through food, water and air borne dust particles[9]. Higher bioavailability of aluminium might manifest as ecotoxicity in general.

With the above views in mind, we have presently studied the aluminium uptake by the seeds under different chemical milieu and effect of aluminium absorption on the seed germination and morphological aspects of germinated seeds of some edible plants *viz*., bengal gram (*Cicer arietinum*), pea (*Pisum sativum*) and horse gram (*Dolichos biflorus*).

EXPERIMENTAL

All chemicals used were of Analytical Reagent grade. Aluminium sulphate [$Al_2(SO_4)_3 \cdot 18\ H_2O$] was used to prepare aluminium ion solution. Calcium chloride ($CaCl_2 \cdot 6H_2O$) was used to prepare solution containing calcium ion. Disodium malate, disodium tartarate and trisodium citrate were used to

prepare solutions containing malate, tartarate and citrate ion, respectively. All the solutions were prepared in distilled water. Different solutions prepared were as follows. (i) Solution of 50 ppm concentration of Al^{3+} ion, (ii) solution of 100 ppm concentration of Al^{3+} ion, (iii) solution of 200 ppm concentration of Al^{3+} ion, (iv) solution of 400 ppm concentration of Al^{3+} ion, (v) Solution of 800 ppm concentration of Al^{3+} ion, (vi) solution of 1000 ppm concentration of Al^{3+} ion, (vii) Mixed solution containing 50 ppm Al^{3+} ion and 50 ppm Ca^{2+} ion concentration, (viii) mixed solution containing 100 ppm Al^{3+} ion and 50 ppm Ca^{2+} ion concentration, (ix) mixed solution containing 200 ppm Al^{3+} ion and 50 ppm Ca^{2+} ion concentration, (x) mixed solution containing 400 ppm Al^{3+} ion and 50 ppm Ca^{2+} ion concentration, (xi) mixed solution containing 800 ppm Al^{3+} ion and 50 ppm Ca^{2+} ion concentration, (xii) mixed solution containing 1000 ppm Al^{3+} ion and 50 ppm Ca^{2+} ion concentration, (xiii) mixed solution of 50 ppm Al^{3+} ion and 50 ppm malate ion concentration, (xiv) mixed solution of 100 ppm Al^{3+} ion and 50 ppm malate ion concentration, (xv) mixed solution of 200 ppm Al^{3+} ion and 50 ppm malate ion concentration, (xvi) mixed solution of 400 ppm Al^{3+} ion and 50 ppm malate ion concentration, (xvii) mixed solution of 50 ppm Al^{3+} ion and 50 ppm citrate ion concentration, (xviii) mixed solution of 100 ppm Al^{3+} ion and 50 ppm citrate ion concentration, (xix) mixed solution of 200 ppm Al^{3+} ion and 50 ppm citrate ion concentration, (xx) mixed solution of 400 ppm Al^{3+} ion and 50 ppm citrate ion concentration, (xxi) mixed solution of 50 ppm Al^{3+} ion and 50 ppm tartarate ion concentration, (xxii) mixed solution of 100 ppm Al^{3+} ion and 50 ppm tartarate ion concentration, (xxiii) mixed solution of 200 ppm Al^{3+} ion and 50 ppm tartarate ion concentration, (xxiv) mixed solution of 400 ppm Al^{3+} ion and 50 ppm tartarate ion concentration.

Seeds of bengal gram (*Cicer arietinum*), pea (*Pisum sativum*) and horse gram (*Dolichos biflorus*) were procured from local market and washed thoroughly with tap water followed by distilled water.

Deep soaking experiments: 50 seeds each of bengal gram, pea or horse gram were placed in 100 mL solutions of 50, 100, 200, 400, 800 and 1000 ppm of aluminium ion (in the form of aluminium sulphate) separately in 250 mL beakers. One blank set for each species of seed containing 50 seeds and 100 mL distilled water (in place of solution) were also set up. Thus a total of 21 sets including three blank sets were set up. The beakers were covered with watch glass and left for 24 h. After 24 h, the volume of the solutions were measured in each set and total aluminium content in the solution in case of each set were determined separately by colorimetric method using Eriochrome Cyanine R reagent[15]. The seeds that were removed from the solutions in the sets were allowed to germinate for 10 days. From time to time, small amount of tap water was sprinkled on the seeds during germination. Percentage germination in each case was determined at the end of 10 days.

Next, in another experiment 50 seeds of each species were again soaked in 100 mL of solutions of different aluminium ion concentrations as above and were left for 48 h. At the end of 48 h, the seeds were removed and allowed to germinate for 10 days and the percentage germination determined at the end of 10 days. The volume of the solutions were measured for each set after removal of seeds. The aluminium content in the total volume of solution was determined[15].

From the aluminium contents of solutions before and after experiments in each case, the aluminium uptake by the total number of seeds were determined. From this, the aluminium uptake per seed was determined in each case.

In another experiment, 50 seeds of each species were deep soaked in 100 mL solution containing different concentrations of aluminium ion and 50 ppm of Ca^{2+} ion (in the form of calcium chloride) for 24 h. At the end of 24 h, the seeds were removed and aluminium content of the solutions were determined (to study the Al uptake by the seeds). The removed seeds were allowed to germinate for 10 days and the percentage germination was determined.

Experiments in shallow soaking: Thirty seeds each of bengal gram, pea and horse gram were placed in petri dishes and were separately treated with 10 mL of solution of different concentration of Al^{3+} ion (in the form of aluminium sulphate). Blank sets for each species of seeds (distilled water in place of solution) were also set up. The sets were left covered (with watch glass) for 24 h. At the end of 24 h, the solutions were found to be almost soaked out and the seeds were left for germination for 10 days. From time to time, tap water was sprinkled onto the seeds during germination. At the end of 10 days, percentage germination in each case was found out. In each case, out of the germinated plants, three well germinated plants were selected and the root length and shoot length of the plants were measured. Mean root length and shoot length were calculated out.

Similar experiments of shallow soaking of 30 seeds of each species were carried out by treating with 10 mL of solutions of different Al^{3+} ion concentration containing 50 ppm Ca^{2+} ions for 24 h, the seeds were allowed to germinate for 10 days. Percentage germination were calculated out and root length and shoot length of three well germinated plants were determined. Mean root length and shoot length were calculated out.

In another experiment, 50 seeds of bengal gram, pea and horse gram were separately soaked in 100 mL of solution containing different Al^{3+} ion concentration (ranging from 100-400 ppm) and 50 ppm concentration of chelating ion *viz.*, malate, citrate or tartarate. After 24 h deep soaking, the seeds were separated out from the solutions in each set and the amount of Al^{3+} ion still present in the solution was measured. From this the amount of aluminium ion absorbed by the seeds was calculated out. After removing from the solutions, the seeds were allowed to germinate for 10 days and percentage germination was noted.

Experiments in soil culture: Ten seeds each of bengal gram, pea or horsegram were sown in 2 kg of soil in eathen pots in a number of sets. The soil in the pots were treated with calculated amount of aluminium sulphate, so that the soil in the pots would have 50, 100, 200, 400, 800 and 1000 ppm Al^{3+} concentration by weight. Blank set in each case (without aluminium ions) were also set up. One set each of different concentration were separately sown with 10 seeds each of all the three species in the same pot. The pots were left in a place where proper sunlight would be available to them during the

day hours. From time to time, proper amount of tap water was added to the pots for providing proper moisture. The germination and growth pattern were observed for 20 days. At the end, three best grown plants in each case were dug out and morphological aspects of the root and shoot *e.g.*, shoot length, number of leaves and nature of roots were recorded. Mean shoot length and number of leaves were calculated out.

RESULTS AND DISCUSSION

Aluminium uptake by different seeds and their percentage germination after deep soaking in solutions of different Al^{3+} ion concentration for 24 and 48 h are recorded in Tables 1 and 2, respectively. Aluminium uptake and percentage germination of seeds after 24 h deep soaking in mixed solutions of different Al^{3+} ion concentration and 50 ppm Ca^{2+} ion concentration are recorded in Table-3. Percentage germination, root length and shoot length of plants of different seeds after 24 h shallow soaking in solutions of different concentrations of Al^{3+} ion are recorded (Table-4). Percentage germination, root length and shoot length of plants of different seeds after 24 h shallow soaking in Ca^{2+} ion mixed with Al^{3+} ion solutions are recorded in Table-5. Percentage germination and aluminium uptake by different seeds deep-soaked for 24 h in solutions of different Al^{3+} ion concentration in presence of chelating agents like malate, citrate or tartarate are recorded in Tables 6-8, respectively. Data concerning morphological aspects of different seeds sown in soil culture are recorded in Tables 9 and 10.

Toxicity of aluminium would primarily be decided by its uptake by the seeds and its affect on germination as well as morphological aspects of plants coming out of germinated seeds. Table-1 suggests that bengal gram (*Cicer arietinum*) seem to be most highly affected in percentage germination with increasing aluminium concentration. This is followed by horse gram (*Dolichos biflorus*) and pea (*Pisum sativum*). At 400 ppm Al^{3+} concentration onwards, the germination in gram is zero indicating high phytotoxicity. In pea and horse gram also, 24 h deep soaking in aluminium solution gradually decreases the percentage germination with increasing aluminium concentration. But it is never zero. So far as aluminium uptake is concerned there has been a gradual increase in the aluminium

TABLE-1
ALUMINIUM UPTAKE BY BENGAL GRAM, PEA AND HORSE GRAM SEEDS FROM THE SOLUTION OF DIFFERENT Al^{3+} CONCENTRATION AFTER 24 h DEEP SOAKING TREATMENT AND PERCENTAGE GERMINATION OF THE SEEDS AFTER 10 DAYS

Al^{3+} Conc. (ppm)	Bengal gram		Pea		Horse gram	
	Percentage germination	Al^{3+} uptake in mg/seed	Percentage germination	Al^{3+} uptake in mg/seed	Percentage germination	Al^{3+} uptake in mg/seed
00 (Blank)	98	–	44	–	80	–
50	90	0.03	40	0.04	70	0.06
100	4	0.06	38	0.17	24	0.10
200	2	0.08	33	0.37	22	0.17
400	0	0.15	30	0.66	21	0.45
800	0	0.46	24	1.33	20	1.31
1000	0	0.60	16	1.77	16	1.65

TABLE-2
ALUMINIUM UPTAKE BY BENGAL GRAM, PEA AND HORSE GRAM SEEDS FROM THE SOLUTION OF DIFFERENT Al^{3+} CONCENTRATION AFTER 48 h DEEP SOAKING TREATMENT AND PERCENTAGE GERMINATION OF THE SEEDS AFTER 10 DAYS

Aluminium conc. (ppm)	Bengal gram		Pea		Horse gram	
	Percentage germination	Al^{3+} uptake in mg/seed	Percentage germination	Al^{3+} uptake in mg/seed	Percentage germination	Al^{3+} uptake in mg/seed
00 (Blank)	94	–	40	–	36	–
50	90	0.03	30	0.07	30	0.06
100	16	0.07	20	0.18	8	0.18
200	2	0.09	15	0.33	4	0.27
400	0	0.16	0	0.68	0	0.50
800	0	0.49	0	1.47	0	1.51
1000	0	0.81	0	1.79	0	1.72

TABLE-3
ALUMINIUM UPTAKE BY BENGAL GRAM, PEA AND HORSE GRAM SEEDS FROM MIXED SOLUTION OF DIFFERENT CONCENTRATION OF Al^{3+} AND 50 ppm Ca^{2+} AFTER 24 h DEEP SOAKING TREATMENT AND PERCENTAGE GERMINATION OF THE SEEDS AFTER 10 DAYS

Al^{3+} conc. (ppm)	Ca^{2+} conc. (ppm)	Bengal gram		Pea		Horse gram	
		Percentage germination	Al^{3+} uptake in mg/seed	Percentage germination	Al^{3+} uptake in mg/seed	Percentage germination	Al^{3+} uptake in mg/seed
00 (Blank)	50	80	–	40	–	40	–
50	50	60	0.03	18	0.04	37	0.05
100	50	54	0.05	12	0.16	16	0.12
200	50	2	0.07	8	0.32	6	0.14
400	50	0	0.14	6	0.67	4	0.42
800	50	0	0.44	4	1.36	0	1.30
1000	50	0	0.58	3	1.81	0	1.68

TABLE-4
PERCENTAGE GERMINATION IN 10 DAYS AND MEAN ROOT AND SHOOT LENGTH AFTER 24 h SHALLOW SOAKING TREATMENT OF SEEDS OF BENGAL GRAM, PEA AND HORSE GRAM IN SOLUTIONS OF DIFFERENT CONCENTRATION OF Al^{3+}

Al^{3+} conc. (ppm)	Bengal gram			Pea			Horse gram		
	Percentage germination	Root length (cm)	Shoot length (cm)	Percentage germination	Root length (cm)	Shoot length (cm)	Percentage germination	Root length (cm)	Shoot length (cm)
00 (Blank)	97	4.6	2.0	93	5.0	2.5	63	4.2	2.8
50	97	3.8	1.4	92	2.4	2.0	33	3.5	2.6
100	95	3.0	1.5	83	2.0	1.4	28	2.1	2.2
200	93	2.8	1.4	66	1.8	1.4	26	2.0	1.8
400	60	2.1	1.0	30	1.6	1.3	25	0.7	1.2
800	16	2.0	0.9	30	1.4	1.2	13	0.7	1.0
1000	8	1.8	0.9	24	1.0	1.2	10	0.3	1.0

TABLE-5
PERCENTAGE GERMINATION IN 10 DAYS AND MEAN ROOT AND SHOOT LENGTH AFTER 24 h SHALLOW SOAKING TREATMENT OF SEEDS OF BENGAL GRAM, PEA AND HORSE GRAM IN MIXED SOLUTION OF DIFFERENT Al^{3+} CONCENTRATION AND 50 ppm Ca^{2+} CONCENTRATION

Al^{3+} conc. (ppm)	Ca^{2+} conc. (ppm)	Bengal gram			Pea			Horse gram		
		Percentage germination	Root length (cm)	Shoot length (cm)	Percentage germination	Root length (cm)	Shoot length (cm)	Percentage germination	Root length (cm)	Shoot length (cm)
00 (Blank)	50	94	2.5	19.0	40	4.2	2.6	42	6.2	4.0
50	50	90	2.0	17.0	32	2.2	2.4	30	3.2	1.8
100	50	56	2.0	16.3	18	2.1	2.4	20	2.8	1.4
200	50	36	2.0	16.0	6	1.5	2.3	6	2.8	1.0
400	50	10	1.9	14.3	3	1.2	2.3	4	0.5	0.7
800	50	4	1.8	13.5	0	–	–	0	–	–
1000	50	4	1.6	12.7	0	–	–	0	–	–

TABLE-6
PERCENTAGE GERMINATION OF SEEDS IN 10 DAYS AND ALUMINIUM UPTAKE BY SEEDS OF BENGAL GRAM, PEA AND HORSE GRAM FROM MIXED SOLUTIONS OF DIFFERENT CONCENTRATION OF ALUMINIUM ION AND 50 ppm MALATE ION AFTER 24 h DEEP SOAK TREATMENT OF SEEDS

Al^{3+} conc. (ppm)	Malate ion conc. (ppm)	Bengal gram		Pea		Horse gram	
		Percentage germination	Al^{3+} uptake in mg/seed	Percentage germination	Al^{3+} uptake in mg/seed	Percentage germination	Al^{3+} uptake in mg/seed
00 (Blank)	50	4	–	0	–	0	–
50	50	10	0.08	0	0.09	0	0.06
100	50	2	0.17	0	0.16	0	0.06
200	50	0	0.31	0	0.29	0	0.37
400	50	0	0.69	0	0.63	0	0.42

TABLE-7
PERCENTAGE GERMINATION OF SEEDS IN 10 DAYS AND ALUMINIUM UPTAKE BY SEEDS OF BENGAL GRAM, PEA AND HORSE GRAM FROM MIXED SOLUTIONS OF DIFFERENT CONCENTRATION OF ALUMINIUM ION AND 50 ppm CITRATE ION AFTER 24 h DEEP SOAK TREATMENT OF SEEDS

Al^{3+} conc. (ppm)	Citrate ion conc. (ppm)	Bengal gram		Pea		Horse gram	
		Percentage germination	Al^{3+} uptake in mg/seed	Percentage germination	Al^{3+} uptake in mg/seed	Percentage germination	Al^{3+} uptake in mg/seed
00 (Blank)	50	12	–	0		15	–
50	50	4	0.08	0	0.09	14	0.06
100	50	3	0.16	0	0.16	12	0.08
200	50	0	0.21	0	0.35	0	0.30
400	50	0	0.57	0	0.56	0	0.64

uptake by the seeds with the increasing aluminium concentration in solution. This is true with all the three seeds *i.e.*, bengal gram, pea and horse gram. However, the aluminium uptake is relatively high in case of pea and horse gram compared to bengal gram. However, despite higher aluminium uptake the toxicity expression is less in case of pea and horse gram. Aluminium seems to be highly toxic for bengal gram because even with low aluminium uptake, its germination has been very poor at higher aluminium ion concentration of solution. Table-2 suggests that there is not much difference in the germination percentage as well as aluminium uptake for 48 h deep soaking as compared to the data for 24 h deep soaking. It looks almost complete saturation of aluminium uptake occurs within 24 h of soaking of seeds and a further increase of time of soaking

TABLE-8
PERCENTAGE GERMINATION IN 10 DAYS AND ALUMINIUM UPTAKE BY SEEDS OF BENGAL GRAM, PEA AND HORSE GRAM FROM MIXED SOLUTIONS OF DIFFERENT CONCENTRATION OF ALUMINIUM ION AND 50 ppm TARTARATE ION AFTER 24 h DEEP SOAK TREATMENT OF SEEDS

Al^{3+} conc. (ppm)	Tartarate ion conc. (ppm)	Bengal gram		Pea		Horse gram	
		Percentage germination	Al^{3+} uptake in mg/seed	Percentage germination	Al^{3+} uptake in mg/seed	Percentage germination	Al^{3+} uptake in mg/seed
00 (Blank)	50	96	–	0	–	32	–
50	50	47	0.05	0	0.08	20	0.05
100	50	16	0.08	0	0.11	8	0.07
200	50	0	0.10	0	0.18	0	0.18
400	50	0	0.56	0	0.22	0	0.65

TABLE-9
PERCENTAGE GERMINATION, MEAN SHOOT LENGTH AND NUMBER OF LEAVES (AT 20 DAYS) OF BENGAL GRAM, PEA AND HORSE GRAM SEEDS SOWN SEPARATELY IN SOIL CONTAINING DIFFERENT CONCENTRATIONS OF Al^{3+}

Al^{3+} conc. (ppm)	Bengal gram			Pea			Horse gram		
	Percentage germination	Shoot length (cm)	No. of leaves	Percentage germination	Shoot length (cm)	No. of leaves	Percentage germination	Shoot length (cm)	No. of leaves
00 (Blank)	100	42	110	100	25	18	90	16	6
50	100	40	96	90	22	12	90	14.5	5
100	95	32	82	90	21.8	12	80	13.1	5
200	92	30.5	81	80	21.5	10	70	12.2	5
400	90	25.5	76	75	19	10	50	10.3	5
800	85	23	71	50	15	6	40	9.0	5
1000	80	20	68	48	12	6	35	8.1	5

TABLE-10
PERCENTAGE GERMINATION, MEAN SHOOT LENGTH AND NUMBER OF LEAVES (AT 20 DAYS) OF BENGAL GRAM, PEA AND HORSE GRAM SEEDS SOWN COMBINDLY IN SOIL CONTAINING DIFFERENT CONCENTRATIONS OF Al^{3+}

Al^{3+} conc. (ppm)	Bengal gram			Pea			Horse gram		
	Percentage germination	Shoot length (cm)	No. of leaves	Percentage germination	Shoot length (cm)	No. of leaves	Percentage germination	Shoot length (cm)	No. of leaves
00 (Blank)	100	46	81	90	26	20	60	12.5	5
50	95	43	78	85	25	20	55	12	5
100	90	32	71	80	20	12	54	11	5
200	80	31	68	70	16	10	50	10.5	5
400	70	30	65	60	15.5	10	44	10	3
800	60	26	54	20	10.5	7	42	9.5	2
1000	55	23	46	20	9.7	7	35	9.2	2

does not increase the uptake anymore. The results in 48 h deep soaking are more or less comparable to that of 24 h, except with a little excess aluminium uptake in some cases. Kinetically, 24 h period seem to be sufficient to express full toxicity.

Present experiments with calcium mixed with Al^{3+} ion solution for 24 h deep soaking of seeds (Table-3) suggest that somehow, Ca^{2+} ions are unable to alleviate aluminium uptake and toxicity. The percentage germination has still been almost comparable (in case of bengal gram) and rather poor (in case of pea and horse gram), to the results with only aluminium ion solution without any calcium. The relative aluminium uptake in presence of Ca^{2+} ion has also almost been similar as compared to that without calcium. Far from alleviating aluminium toxicity the calcium ion, somehow, itself expresses some toxicity to pea and horsegram seeds. It might also be that calcium itself is not creating toxicity but somehow, pushes more of aluminium into the seeds to express higher toxicity. This is because in some cases there is slightly higher aluminium uptake by seeds compared to that without calcium. Calcium might be chelating with some important complexones released by the seeds which otherwise would have chelated with aluminium and prevented its uptake.

The experiments with shallow soaking of seeds (Tables 4 and 5) suggest that the percentage germination is only slightly affected upon shallow soaking of seeds. At higher concentration of aluminium, however, the toxicity is definitely expressed and the percentage germination rapidly falls from 400 ppm onwards. The toxicity is severely expressed in case of bengal gram, once again. The root length and the shoot length of the germinated plants have been found to be decreased with increasing Al^{3+} concentration. It is seen that the roots are more severely affected than the shoots. In shallow soaking, the contact of seeds with the solution as well as the total volume of the solution being very less compared to that in deep soaking, the affect of aluminium is surely likely to be less in this case (shallow soaking) as compared to that in deep soaking. This has actually been observed. It is seen from Table-5 that in presence of 50 ppm Ca^{2+} ion, the aluminium toxicity in shallow soaking also is not alleviated much. Rather calcium ions have adversely affected the germination percentage in case of pea and horsegram. The root and shoot length of the germinated plants have almost remained comparable to those germinated in only aluminium ion soaking. Thus, once again it is observed that the theory of alleviation of aluminium toxicity by Ca^{2+} ion somehow is not substantiated[16,17].

Metal detoxification by chelating agent has been useful method for controlling metal toxicity. Presently, we have studied simple chelating organic acids as inhibitors of aluminium uptake by the seeds. These simple organic acids might either inhibit the metal uptake by chelating aluminium ions or themselves chelate out preferably with some useful metal of seeds and become toxic to the seeds on one hand and help out aluminium uptake by the useful-metal holding chelators of seeds which have now been set free by the release of their useful metals, thus creating more and more toxicity, on the other hand.

Presently, it is seen from Table-6, that the malate ion itself exhibits high toxicity to the process of germination at 50 ppm concentration. The germination percentage was found only 4 % for bengal gram and 0 % germination for pea and horse gram. In presence of aluminium (50 ppm) the malate (50 ppm) shows a little amelioration of toxicity, only for bengal gram but not for pea and horse gram. At still higher concentration of aluminium, the toxicity of malate and aluminium seem to become additive, thus causing a high toxicity. In fact for pea and horse gram, the malate and aluminium combination resulted in complete inhibition of germination at all concentrations of aluminium. The reason behind malate toxicity might be that it is extracting out (by chelation) some useful indispensable metal ions of the seeds, thus causing breakdown of germination process. The chelating agent inside the seeds which probably get released after removal of its useful metal constituent might now be uptaking aluminium ions (through chelation) present in the vicinity. This is substantiated from the fact that though malate and aluminium combination inhibits germination but is unable to prevent aluminium uptake by the seeds. There has been a uniform increase in aluminium uptake with increasing Al^{3+} ion concentration in the milieu.

Study of Table-7 shows that even the citrate ion some how has become incapable of ameliorating aluminium toxicity. More or less the citrate behaved just like malate. However, the pure citrate ion showed a little less toxicity compared to malate. There was 12 % germination for bengal gram and 15 % germination for horse gram in presence of citrate without aluminium. In presence of citrate and aluminium combination, the toxicity, once again, increased, with very poor germination, except for horse gram where the germination was slightly better upto 100 ppm Al^{3+} concentration. The aluminium uptake, as usual, increased with increasing Al^{3+} in the milieu. Thus it seems citrate and aluminium toxicities become additive and do not counteract each other. Combindly, they rather enhance the toxicity.

So far as tartarate is concerned (Table-8), it seems to be relatively less toxic by itself for bengal gram and horse gram. When singly present at 50 ppm concentration, it shows upto 90 % germination for bengal gram and 32 % for horse gram. This indicates that by itself it is not toxic at least for bengal gram. In combination with aluminium (50 ppm) there has been better germination (47 % for bengal gram and 32 % for horse gram) and low aluminium uptake as compared to the similar cases with citrate and malate as chelating agent. For pea, however, the tartarate alone or in combination with aluminium was found to be totally toxic exhibiting zero germination at all concentrations. At higher concentration of aluminium *i.e*., 200 ppm and above even bengal gram and horse gram seeds failed to show any germination. Thus, it seems tartarate as a chelating agent is itself not much toxic and could alleviate aluminium toxicity at low Al^{3+} concentration for bengal gram and horse gram. In any case our results with chelating agents suggest that, somehow, they are unable to ameliorate aluminium toxicity to a significant extent.

Results of present experiments in soil culture (Table-9) suggested some morphogenic toxicity of Al^{3+} ions in soil. Though the germination percentage was not much affected, particularly for bengal gram, with increasing aluminium concentration in soil, the morphological aspects such as shoot length, number of leaves as well as the nature of roots got severely affected. At higher Al^{3+} concentration, the degeneration of plants started 10 days after growth. The leaves started curving out and plants looked lifeless, more so in case of pea. The rate of growth of the plants was quite poor at higher aluminium concentration. The plants had many adventitious roots but primary root was quite short at Al^{3+} concentration above 400 ppm.

In mixed culture in soil (all the three plant species in the same pot) it is the horse gram plants which was badly affected. Its percentage germination in blank as well as aluminium toxicated soil was found to be quite less compared to those of bengal gram and pea (Table-10). It looks resistance to aluminium toxicity is quite less in case of horse gram in comparison to bengal gram and pea. Even in mixed culture, these plants showed degenerative disposition and lifelessness at aluminium concentrations above 400 ppm. Thus, it seems even in soil culture the aluminium toxicity would prevail for plants; but quite expectedly, to a lesser extent than the plants germinated from seeds soaked in aluminium ion solutions directly.

Conclusion

The present studies suggest that Al^{3+} ions are quite toxic to the germination and growth of seeds and plants of bengal gram, pea and horse gram. The process of germination get severely affected in solution culture containing Al^{3+} above 200 ppm. Even in soil culture, the percentage germination as well as morphogenic aspects of plants get severely affected by aluminium toxicity. In presence of mixture of seeds of the three species (bengal gram, pea and horse gram) there seems to exist some preference in the exhibition of toxicity. Horse gram seems to loose the race and thus exhibit higher aluminium toxicity and less resistance compared to bengal gram and pea. Thus, finally, it can be concluded that factors causing aluminium ion release from soil to the ground water, such as acid rain, acidic industrial affluent or direct increase of aluminium load in the soil, water, or air through pollution/mining activities *etc*., should be checked/ controlled and proper remedial measures should be adopted to avoid the ecotoxicity of aluminium and consequently, prevent the aluminium toxicity transmission through the edible plants.

REFERENCES

1. J.M. Candy, J. Klinowski, R.H. Perry, E.K. Perry, A. Fairbairn, A.E. Oakley, T.A. Carpenter, J.R. Atack, G. Blessed and J.A. Edwardson, *Lancet*, **327**, 354 (1986).
2. H. Sigel and A. Sigel, Aluminium and its Role in Biology In: Metal Ions in Biological Systems, New York: Marcel Dekker, Vol. 24, p. 424 (1988).

3. C. Exley, J.S. Chappell and J.D. Birchall, *J. Theor. Biol*., **157**, 417 (1991).
4. C. Exley and J.D. Birchall, *J. Theor. Biol*., **159**, 83 (1992).
5. P. Zatta, *Trace Elem. Med*., **10**, 120 (1993).
6. A.C. Alfrey, R. Garg, G.R. Legendra and W.D. Kochhy, *N. Engl. J. Med*., **294**, 184 (1994).
7. A.C. Alfray, *Life Chem. Rep.(S)*, **11**, 197 (1994).
8. C.R. Harrington, C.M. Wischik, F.K. McArthur, G.A. Taylor, J.A. Edwardson, J.M. Candy, *The Lancet*, **343**, 993 (1994).
9. P. Zatta, *Med. Hypoth*., **44**, 169 (1995).
10. P. Nayak and A.K. Chaterjee, *J. Toxicol. Sci*., **23**, 1 (1998).
11. P. Nayak and A.K. Chaterjee, *J. Environ. Biol*., **20**, 77 (1999).
12. P. Nayak and A.K. Chaterjee, *Food Chem. Toxicol*., **39**, 587 (2001).
13. P. Nayak, *Environ. Res. Sec. A*, **89**, 111 (2002).
14. P. Nayak and A.K. Chaterjee, *BMC Neuro. Sci*., **3**, 12 (2002).
15. A.I. Vogel, A Text Book of Quantitative Inorganic Analysis, E.L.B.S. and Longman, London, edn. 4, p. 729 (1978).
16. P.R. Ryan, T.B. Kinraide and L.V. Kochian, *Planta*, **192**, 98 (1994).
17. P.R. Ryan, J.R. Robert and F.A. Smith, *Plant Physiol*., **113**, 1351 (1997).