Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2014, 4(4):126-130

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Effect of some inorganic and organic anions on aluminium toxicity to a fresh water fish, *Channa punctatus*

T. V. R. K. Rao* and Arjun Kumar

Department of Chemistry, Purnea College, Purnia(Bihar), India

---

ABSTRACT

Effect of some inorganic and organic anions viz., fluoride, silicate, phosphate, malate, tartrate or citrate on aluminium toxicity to an edible fresh water fish, Channa punctatus, has been studied in aquarium models. 10 fish in each case were added to different aquariums consisting of 0.001M Al^{3+} solutions, along with different inorganic/organic anions (fluoride, silicate, phosphate, malate, tartrate or citrate) in separate sets. One aquarium contained only Al^{3+} and no other anions. Similar number of fish were also studied in Control set. The fish in all the sets were exposed to toxicity till 60 days or total mortality, whichever was earlier. Mortality of the fish was recorded. At the end, the fish that died last in case of sets where total mortality was observed and surviving fish at 60 days in case of sets in which the fish survived till last, were sacrificed. Five fish of Control set were also sacrificed. Aluminium uptake by the tissue of the fish in different parts viz., head, middle and tail part were studied. Results revealed that the exposure of the fish to aluminium concentration of 0.001M is chronically toxic. 80% of the fish died gradually in 60 days. The upper part of fish (brain and gills) were found to be the major sites of aluminium accumulation. The effect of inorganic and organic anions on aluminium uptake as well as fish mortality were found to be different for different anions. All the anions inhibited the aluminium uptake by the fish tissue as compared to that in aluminium alone exposed set. The inhibition of Al uptake by the anions has been in the range of 59.58 to 84.58%. However, their effect on mortality of the fish were different. Fluoride and malate rather proved to be promoter of mortality as compared to Al^{3+} alone set. Silicate, phosphate, tartrate and citrate proved to be inhibitors of mortality also. Inhibition of mortality by silicate was found to be 37.5%. Phosphate, tartrate and citrate inhibited Al exposed fish mortality by as high as 87.5%. The results suggest that fluoride and malate might be exhibiting their own toxicity in addition to Al toxicity. Phosphate, tartrate and citrate were found to be good inhibitors of Al uptake by the fish, as well as, their mortality.

Key words: Aluminium toxicology, Aluminium ecotoxicology, Aluminium toxicity to fish, Aluminium uptake. Aluminium uptake inhibition by anions.

INTRODUCTION

Aluminium toxicity to fauna and flora has recently been finding much interest. This is because aluminium exposure has been reported to be neurotoxic [1-14]. Bioavailability of aluminium is limited, despite its heavy content in the soil. This is because aluminium in the soil is present as complex alumino silicates, which are quite stable. However, in the event of acid rain and other pH lowering factors, there may be leaching of aluminium to the ground water in the soluble form, resulting in toxicity to the living kingdom. Aluminium toxicity to the fish has been reported earlier [15-22]. Studies on aluminium toxicity in the fish would serve as effective models for studying aluminium neurotoxicity in humans.

Aluminium uptake and hence its toxicity might be affected by the presence of different inorganic or organic anions in the milieu. Some of the naturally occurring inorganic/organic anions might prove to be ameliorator of Al toxicity. Such studies would have applied value in the prevention/minimization of Al toxicity.

With the above view in mind, we have presently studied on the effect of some inorganic and organic anions on aluminium toxicity to a fresh water fish, *Channa punctatus*.

MATERIALS AND METHODS

All chemicals used were of A.R. (Analytical Reagent) quality. Aluminium sulphate, [$Al_2SO_4.16H_2O$], was used to prepare aluminium ion (Al^{3+}) solution. Sodium fluoride, sodium silicate, sodium phosphate, di-sodium malate, di-sodium tartrate and tri-sodium citrate were separately used to prepare solutions containing fluoride (F^-), silicate (SiO_3^{2-}), phosphate (PO_4^{3-}), malate ($C_4H_4O_5^{2-}$), tartrate ($C_4H_4O_6^{2-}$) and citrate ($C_6H_5O_7^{3-}$) ions respectively.

Channa punctatus fish were procured from the local market and were reared in an aquarium in fresh water under laboratory conditions. The fish were allowed to acclimatize to the aquarium conditions for one week. After one week, 10 fish each were placed in eight aquariums of similar dimension and capacity (40 litre water). The aquariums were charged with water/solutions as follows:

1. 40 L water (Control set).
2. 40 L water + calculated quantity of aluminium sulphate (for 0.001M).
3. 40 L water + calculated quantities of aluminium sulphate (for 0.001M) & sodium fluoride (for 0.001M).
4. 40 L water + calculated quantities of aluminium sulphate (for 0.001M) & sodium silicate (for 0.001M).
5. 40 L water + calculated quantities of aluminium sulphate (for 0.001M) & sodium phosphate (for 0.001M).
6. 40 L water + calculated quantities of aluminium sulphate (for 0.001M) & di-sodium malate (for 0.001M).
7. 40 L water + calculated quantities of aluminium sulphate (for 0.001M) & di-sodium tartrate (for 0.001M).
8. 40 L water + calculated quantities of aluminium sulphate (for 0.001M) & tri-sodium citrate (for 0.001M).

The fish in the aquarium were fed with fish food at stipulated time during the day. Almost equivalent quantity of food was given to all the eight sets. Water in the aquarium was well aerated throughout. The health as well as mortality (if any) of the fish were noted at a stipulated time, every 24 hours till 60 days or total mortality time, whichever was earlier. Dead fish were immediately removed out from the aquarium. pH of the aquarium water was also noted from time to time. The pH of Al alone set (0.001M Al^{3+}) and Experimental sets, containing inorganic/organic anions were found to vary in the range of 6.0 to 6.5 during experimentation. The solutions (Experimental sets)/water (Control set) were changed every 20 days. The observation was continued upto 60 days.

At the end, the fish that died last in case of Al^{3+} alone set, Al^{3+} + fluoride set, Al^{3+} + malate set and five surviving fish from each of the Experimental sets viz., Al^{3+} & silicate, phosphate, tartrate or citrate as well as Control set (after sacrificing) were chopped into three parts viz, head part, middle part and the tail part. Each part of the fish was weighed out and treated separately with 10 ml of 1M HNO_3 solution in a conical flask and boiled for 15 minutes, where upon the entire tissue got dissolved. It was further evaporated to dryness. Next, the dry residue was extracted with distilled water and quantitatively filtered into 100 ml volumetric flask. The solution was made upto the mark (100 ml) with the help of distilled water. Aluminium content of the solution was estimated Spectrophotometrically using Eriochrome Cyanine R reagent [23]. The content of aluminium in mg/g of the tissue in the different parts of the fish of the Experimental and Control sets were calculated out separately.

The entire work was carried out in three replicates, and mean aluminium uptake by the different parts of the fish were calculated out.

% Inhibition/promotion of mortality (of the fish) by inorganic/organic anions as compared to Al-alone set has been calculated as per the following formula:

$$\%\ \text{inhibition/promotion of mortality} = \frac{\text{Mortality in Blank set} - \text{Mortality in Experimental set}}{\text{Mortality in Blank set}}$$

- ve value of inhibition has been considered as promotion.

% inhibition of Al uptake (by the fish tissue) by inorganic/ organic anions as compared to Al -alone set has been calculated as per the following formula:

$$\% \text{ inhibition of Al-uptake} = \frac{\text{Al-uptake in Blank set} - \text{Al-uptake in Experimental set}}{\text{Al-uptake in Blank set}}$$

Where,
Blank set = Al^{3+} alone set.
Experimental set = Al^{3+} + inorganic/organic anion set.

Norms of the Institutional Committee for ethics in animal experimentation were strictly followed during the experimentation.

RESULTS AND DISCUSSION

Mortality of the fish (*Channa punctatus*) under different chemical milieu is recorded in Table-1. Mean aluminium uptake by the fish (*Channa punctatus*) under different chemical milieu is recorded in Table-2.

Aluminium has been found to be chronically toxic to the fish (*Channa punctatus*) at an exposure concentration of 0.001M. As seen from the results (Table-1) a total of 80% mortality occurred in 60 days. The onset of mortality started after 30 days and continued to increase rapidly. In the Control set all the fish remained alive and healthy with 0% motality. Effect of the presence of other inorganic and organic anions in the milieu of Al exposed fish, have been found to be varied. In case of fluoride and malate, there has been promotion in the mortality rate. In case of fluoride, all the fish died within first 96 hours. It looks fluoride itself expressed sever toxicity and added further to Al toxicity. Aluminium and fluoride did not negate each other's toxicity and rather multiplied it. In case of malate also, the results were same and there has been a promotion of mortality by 25% as compared to Al alone toxicity. In case of malate, however the onset of toxicity was found to be rather slow (compared to fluoride), the fish gradually died by 10 days. It looks malate itself either induces a rapid Al uptake by the fish or it (malate) itself expresses toxicity to the fish. In case of silicate plus Al exposure, there had been 37.5% inhibition of mortality as compared to Al alone exposure. Only five fish died in a span of 60 days. It looks silicate itself does not exhibit much toxicity and rather controls Al toxicity to some extent. The anions like phosphate, tartrate or citrate, when present in the milieu along with Al^{3+}, have been found to inhibit the mortality rate of the fish to a much greater extent. There had been 87.5% inhibition of mortality by these anions, as compared to Al^{3+} alone exposure.Thus, so far as mortality of the fish is concerned phosphate, tartrate or citrate seem to be good ameliorator of mortality, and hence Al toxicity.

As regards aluminium uptake by the fish tissue is concerned, in all the Experimental sets (Al^{3+} + inorganic/organic anion exposure), there has been inhibition of Al uptake compared to that of Al alone exposure (Table-2). The head part of the fish have been found to be the main target of aluminium uptake. The tail parts uptook relatively very less amount of aluminium. It seems the brain and gill cells have some special affinity for Al^{3+} ions. Accumulation of Al^{3+} in the gills must be intoxicating the respiratory tract, which results in the death of the fish. Aluminium also seems to be producing neurotoxicity, as the fish became mostly inactive much before their death. In the aluminium alone exposure set, the total Al uptake was found to be 4.80 mg/g. Presence of inorganic/organic anions in the milieu along with Al has resulted in a decreased uptake of Al by the fish tissue (Table-2). Fluoride and malate have inhibited the Al uptake by 59.58 and 62.91% respectively, as compared to that of Al alone exposure. Silicate, phosphate, tartrate and citrate have inhibited the Al uptake in the range of 80.41 - 84.58%. The highest inhibition was observed by tartrate (84.58%).

Thus, if the effects of anions on the Al uptake by the fish, as well as on their mortality, are considered combinedly, the fluoride and malate showed abnormal results. Both of these anions inhibited the Al uptake but promoted the mortality. Thus, it seems in the presence of fluoride or malate, the fish mortality was due to the expression of toxicity by fluoride/malate themselves rather than Al toxicity. On one hand, they control Al toxicity to a good extent but themselves individually become toxic and cause mortality. The anions like silicate, phosphate, tartrate and citrate, however, could inhibit the Al uptake, as well as mortality of the fish, both. In case of silicate, however, a high inhibition of Al uptake (80.41%) does not reflect proportionally in the control of fish mortality. It might be due to the fact that silicate inhibits the Al uptake and hence controls Al toxicity, but expresses its own toxicity (silicate toxicity) to some extent at this concentration (0.001M). Phosphate, tartrate and citrate, on the other hand, have inhibited both, Al uptake as well as fish mortality, to a good extent. It seems these anions prevent Al uptake and hence control Al toxicity, and do not exhibit any toxicity of their own. Hence, these anions (phosphate, tartrate and citrate) can be taken as non toxic for the fish and also can be considered as good inhibitors/ameliorators of Al toxicity. The mechanism of action behind the inhibition of Al toxicity by these anions might be through the formation of stable complexes/chelates of aluminium and thus preventing its (aluminium) uptake.

Table-1 Mortality of the fish (*Channa punctatus*) under different chemical milieu

S. No.	Chemical milieu (conc^n. of Al^{3+} = 0.001M &conc^n. of inorganic /organic anion = 0.001M)	Mortality of the fish							Total mortality (60 days)	% mortality	% Inhibition/ promotion of mortality by inorganic/organic anion as compared to Al alone set
		0-4 days	4-10 days	10-20 days	20-30 days	30-40 days	40-50 days	50-60 days			
1.	Water (Control set)	—	—	—	—	—	—	—	—	0	—
2.	Al^{3+}	—	—	—	—	1	4	3	8	80	—
3.	Al^{3+} + fluoride	10	—	—	—	—	—	—	10	100	25.0 (promotion)
4.	Al^{3+} + silicate	—	2	—	2	—	1	—	5	50	37.5 (Inhibition)
5.	Al^{3+} + phosphate	—	1	—	—	—	—	—	1	10	87.5 (Inhibition)
6.	Al^{3+} + malate	—	10	—	—	—	—	—	10	100	25.0 (promotion)
7.	Al^{3+} + tartrate	—	—	—	—	—	1	—	1	10	87.5 (Inhibition)
8.	Al^{3+} + citrate	—	—	—	1	—	—	—	1	10	87.5 (inhibition)

Table-2 Mean aluminium uptake by the fish (*Channa punctatus*) under different chemical milieu

S. No.	Chemical milieu (conc^n. of Al^{3+} = 0.001M & conc^n. of inorganic /organic anion = 0.001M)	Al uptake by the fish tissue (mg/g)				% inhibition of Al uptake by inorganic/ organic anion as compared to Al alone set
		Head part	Middle part	Tail part	Total Al uptake	
1.	Water (Control set)	0.00	0.00	0.00	0.00	__
2.	Al^{3+}	1.80	1.63	1.37	4.80	__
3.	Al^{3+} + fluoride	0.82	0.72	0.40	1.94	59.58
4.	Al^{3+} + silicate	0.41	0.33	0.20	0.94	80.41
5.	Al^{3+} + phosphate	0.38	0.33	0.23	0.94	80.41
6.	Al^{3+} + malate	0.84	0.66	0.28	1.78	62.91
7.	Al^{3+} + tartrate	0.35	0.27	0.12	0.74	84.58
8.	Al^{3+} + citrate	0.35	0.28	0.18	0.81	83.12

CONCLUSION

Our present studies suggest that exposure of Al^{3+} at a concentration of 0.001M is chronically toxic to the fish (*Channa punctatus*). 80% of the fish died gradually by 60 days. The mortality started at 30 days onwards. It looks an effective amount of aluminium uptake by the fish has to be there before it becomes fatally toxic. Our studies also suggest that the presence of fluoride or malate ions vis a vis aluminium in the milieu inhibit the Al uptake by the fish tissue on one hand and promote the mortality rate on the other hand. It seems these anions (fluoride and malate) exhibit their own toxicity to the fish which adds up to the Al toxicity, thus promoting total toxicity. Thus, theses two anions (fluoride and malate) can be considered as promoter of toxicity to the fish, when present with aluminium. Silicate, phosphate, tartrate or citrate anions, when present together with Al^{3+} proved to be inhibitors of aluminium uptake by the fish and also inhibitors of their mortality. As such, these anions, particularly phosphate, tartrate or citrate can be used as inhibitors/ameliorators of Al toxicity to the fish.

REFERENCES

[1] Candy J M, Klinowski J, Perry R H, Perry E K, Fairbairn A, Oakley A E, Carpenter T A, Atack J R, Blessed G and Edwardson J A, *Lancet*, **1986**, 327, 354.
[2] Sigel H and Sigel A, New York: *Marcel Dekker*, **1988**, 24, 424.
[3] Exley C, Chappell J S and Birchall J D, *J Theor Biol* , **1991**, 151, 417.
[4] Exley C and Birchall J D, *J Theor Biol* , **1992**, 159, 83.
[5] Zatta P, *Trace Elem Med* , **1993**, 10**,** 120.
[6] Alfrey A C, LeGendra G R and Koehny W D, *N Engl J Med* , **1976**, 294, 184.
[7] Alfrey A C, *Life Chem Rep* (S), **1994**, 11**,** 197.
[8] Harrington C R, Wischik C M, McArthur F K, Taylor G A, Edwardson J A and Candy J M, *Lancet*, **1994**, 343**,** 993.
[9] Zatta P, *Med Hypoth* , **1995**, 44, 169.
[10] Nayak P and Chaterjee A K, *J Toxicol Sci* , **1998** , 23, 1.
[11] Nayak P and Chaterjee A K, *J Environ Biol* , **1999**, 20**,** 77.
[12] Nayak P and Chaterjee A K, *Food Chem Toxical* , **2001**, 39, 587.
[13] Nayak P, *Environ Res Sec A*, **2002**, 89, 111.
[14] Nayak P and Chaterjee A K, *BMC Neuro Sci* , **2002**, 3**,** 12.
[15] Howells G, Dalziel T R K, Reader J P and Solbe J F, *Chemistry and Ecology* , **1990,** 4**,** 117.
[16] Spry D J and Wiener J G, *Environ Pollut* , **1991**, 71**,** 243.
[17] Karatas M, Seker Y and Sezer M, *Asian J Chem* , **2007,** 19, 574.

[18] Karatas M and Seker Y, *Asian J Chem* , **2008**, 20, 3310.
[19] Karatas M, *Asian J Chem* , **2008**, 20, 5741.
[20] Exley C and Struthers W, *Aquaculture*, **1992,** 100, 323.
[21] Rao T V R K, Yadav Archana and Mishra Kumari Kanchan, *Asian J Chem* , **2013**, 25, 3497.
[22] Rao T V R K and Kumar Arjun, *Cent Euro J Exp Bio* , **2014**, 3(1), 5.
[23] Vogel A I, *A Text Book of Quantitative Inorganic Analysis*, E L B S and Longman, London, **1978**, edn 4, pp 729.