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Aluminium and the effects of chelating agents on liver and brain of *cirrhinus mrigala*

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ABSTRACT

The study of biological indicator organism is more important than analyzing water or sediments for monitoring heavy metal pollution in the aquatic environment .Non-essential elements enter the animals and accumulate to the different organs so that chelating agents are most versatile and effective antidotes for eliminate the metals toxicities. The aim of our present study is to find out bioaccumulations of aluminium and the effects of chelating agents DFO and DFP in brain and liver tissues of Cirrhinus mrigala by using Inductively Coupled Atomic Emission Spectrometer (ICP-AES). This study finds out the accumulation of aluminium is Brain> Liver. The present result suggests that DFO and DFP reduce the aluminium concentration in the tissues of Cirrhinus mrigala fingerlings and both are efficient chelators. Aluminium toxicity is a wide spread problem in all forms of life, including humans, animals, fishes, plants, and cause wide spread degradation of the environment and health.

Keywords: Bioaccumulation, aluminium, chelating agents, ICP-AES.

INTRODUCTION

The estimation of aluminium concentration in the samples of fish has made using an ICP-AES (ISA JOBIN YVON 24 MODAL) and the analytical standard has prepared from the aluminium stock solution. Metal contamination from laboratory was avoided and triplicates of each sample were analysed. The metal concentration in different tissue samples have calculated as follows [1], [2].

Metal concentration (µg/g) = [ICP-AES reading (µg/ml)/sample mass (µg)]x samples volume(ml).

Bio concentration of chemicals by aquatic biota is an important factor in the assessment of the potential hazard of chemicals to the environment. The bio concentration factor or Biological Magnification Factor (BMF) is calculated as BMF = K_1/K_2 =Chemical concentration in each part of the fish (µg/g wet weight)/ Chemical concentration in water (µg/l). Calculation of excretion rate constant chemicals from the whole fish body/organ is

$$C = C_0 e^{-k_2 t}$$

Where, C = Chemical concentration in whole body/ organ (µg/g wet weight) at time t.
C_0= Initial chemical concentration in whole fish body/ organ (µg/g wet weight).
K_2= Excretion rate constant (h^{-1}) and t= time (h^{-1}).

Aluminium, which is the most abundant metal and comprises about 8% of the Earth's crust, is found in combination with oxygen, silicon, fluorine and other elements in the soil, rocks, clays and gems [3]. It has no known biological function [4]. Presently, aluminum utensils are widely used throughout the world, especially in developing countries [5]. The use of such tools may increase an individual's aluminium exposure, particularly when these are used with salty, acidic or alkaline foods [6]. Additionally, aluminium and its salts are commonly used in daily life as it is

believed that it is a non-toxic and is quickly excreted in the urine. However, this element can have negative impact human and animal health [7]. Aluminium is potentially toxic to humans. The Agency for Toxic Substances and Disease Registry (ATSDR) [8], reported that aluminium is distributed mainly in the bone, liver, testis, kidneys and brain. In patients on dialysis [9] or on long-term total parenteral nutrition [10] this metal accumulates in different organs. The toxicological effects of aluminium in humans include encephalopathy [11] bone disease, anaemia and skeletal system disease [12]. Furthermore, aluminium is possibly a contributing factor in the development of Alzheimer's disease [13]. However, this remains contradictory [14], [15]. The chelating agents possess the common ability to form complexes with heavy metals and thereby prevent or reverse the binding of metallic captions to body legends. Chelating therapy is recommended for heavy metal poisoning. Heavy metals exert their toxic effects by combining with one or more reactive groups (legends) essential for normal physiological functions. Deferroxamine (DFO) and Deferiprone (DFP) Chelating agents are designed specifically to compete with these groups for the metals and thereby prevent or reverse toxic effects and enhance the excretion of metals. DFO is the principal product of the various side amines obtained from streptomyces Pilosus [16]. Inductively Couple Plasma Atomic Emission spectroscopy (ICP-AES) is an important technique to study the trace elements at molecular level in various biological samples. It is a valuable technique due to its high sensitivity for detecting the major trace elements [17].

EXPERIMENTAL SECTION

Chemicals

All the chemicals, Aluminium sulphate ($Al_2 (SO4)_3$), Deferrioxamine (DFO) and Deferiprone (DFP) were purchased from S.D. Fine, Novartis and Sigma, chemicals limited, Mumbai, India.

Experimental design

Test specimens were divided in to eleven groups each consisting of 20 fishes and stokes in 20 litre plastic aquaria in Annamalai University, Tamilnadu-608002.

Group-I: Fingerlings treated with metal free water.
Group-II: Fingerlings intoxicated with 17.3 ppm of $Al_2 (SO4)_3$ for 14 days.
Group-III: (i) Fingerlings intoxicated with 17.3 ppm of $Al_2 (SO4)_3$ for 14 days (acute) and again treated with DFO (5mg/kg b.wt.) for another 7 days.
(ii) Fingerlings intoxicated with 17.3 ppm of $Al_2 (SO4)_3$ for 14 days (acute) and again treated with DFP (5mg/kg b.wt.) for another 7 days.
Group-IV: (i) Fingerlings intoxicated with 5.2 ppm of $Al_2 (SO4)_3$ for another 15 days (chronic)
Group-V: Fingerlings intoxicated with 5.2 ppm of $Al_2 (SO4)_3$ for 15 days and again treated with DFO (5mg/kg b.wt.) for another 15 days.
(ii) Fingerlings intoxicated with 5.2 ppm of $Al_2 (SO4)_3$ for 15 days and again treated with DFP (5mg/kg b.wt.) for another 15 days.
Group-VI: Fingerlings intoxicated with 5.2 ppm of $Al_2 (SO4)_3$ for 30 days (chronic).
Group-VII: Fingerlings intoxicated with 5.2 ppm of $Al_2 (SO4)_3$ for 30 days and again treated with DFO (5mg/kg b.wt.) for another 15 days.
(ii) Fingerlings intoxicated with 5.2 ppm of $Al_2 (SO4)_3$ for 30 days and again treated with DFP (5mg/kg b.wt.) for another 15 days.
Group-VIII: Fingerlings intoxicated with 5.2 ppm of $Al_2 (SO4)_3$ for 60 days (chronic).
Group-IX: (i) Fingerlings intoxicated with 5.2 ppm of $Al_2 (SO4)_3$ for 60 days and again treated with DFO (5mg/kg b.wt.) for another 15 days.
(ii) Fingerlings intoxicated with 5.2 ppm of $Al_2 (SO4)_3$ for 60 days and again treated with DFP (5mg/kg b.wt.) for another 15 days.
Group-X: Fingerlings intoxicated with 5.2 ppm of $Al_2 (SO4)_3$ for 90 days (chronic).
Group-XI: (i) Fingerlings intoxicated with 5.2 ppm of $Al_2 (SO4)_3$ for 90 days and again treated with DFO (5mg/kg b.wt.) for another 15 days.
(ii) Fingerlings intoxicated with 5.2 ppm of $Al_2 (SO4)_3$ for 90 days and again treated with DFP (5mg/kg b.wt.) for another 15 days.

Lethality studies

Experiments were conducted in the laboratory for 90 days in 20 liters plastic trough. Unchlorinated water (pH is 8.2, alkanity is 408 mg/L, temperature 27±2 ^{o}c) was used as the test medium. *Cirrhinus mrigala* fingerlings of 4±1 cm and body weight 8±1 gm were used as testing organism. The fish specimens collected from the local pond were acclimatized in the laboratory condition for 90 days [18]. Median lethal concentration (LC_{50}) for 120 hours was determined by the method of Litchfield and Welcoxon (1949).The sub-lethal concentration of aluminium sulphate was prepared on the basis of ten times dilution of the LC_{50} value. Except control group all others groups were again treated with chelating agent DFO and DFP for another 15 days subsequently. All the control fingerlings were fed daily with oil less groundnut cake. End of the experimental period, subjected to estimate the aluminium in Brain, Liver tissues of *Cirrhinus mrigala* by using ICP-AES.

Sample preparation

Dissecting the *cirrhinus mrigala* and taken out the Muscle, gill, kidney, brain and liver. Then the samples were dried at 80 ^{0}c for 24 hours. Then the samples were filled with 2N HNO3 overnight and rinsed several times with double distilled water. The samples were digested by weighing one gram of the sample into a 100 mL Borasil flask and then adding 15 ml of concentrated HNO_3 (55%) and 5ml of concentrated Perchloric acid (70%). Digestion was performed on a hot plate at 80 to 90 ^{0}c for approximately 120 minutes or until the solutions become dried. After the digestion [19] was completed, each sample was allowed to cool before being filter through a whatman No. 42 filter paper using vacuum pump. After filtration, the filtering system was rinsed with distilled water to remove all trace

elements, and each sample was made up to 25 mL with distilled water and stored in acid-washed polyethylene flasks until the metal concentration analysis.

Statistical Analysis:

Statistical analysis was performed using SPSS software program, version 11.5. The results were expressed as mean ± standard deviations. The data were analyzed by analysis of variance (ANOVA). A Probability level (*p*-value) of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Brain

From our study we found that the brain tissue accumulates the aluminium 98.23 µg/g in acute and for chronic exposure 18.48 µg/g, 48.53µg/g, 68.71 µg/g, 106.63µg/g for 15, 30, 60, 90 days respectively as shown in table-1. Treatment with the chelating agents DFO and DFP reduced the concentration of aluminium in this tissue to significantly 72% and 63% (acute), 55% to 74% and 49% to 64% (chronic) as shown in Fig-1.The uptake rate increases suddenly upto 60^{th} day and slowly afterwards upto 90^{th} day as shown in Fig-2. The excretion rate decrease suddenly up to 60^{th} day and slowly decrease upto 90^{th} day as shown in Fig-3. The BMF increases in the initial period and greatest bioconcentration factor occurred at lowest exposure level (5.2 ppm) for 90^{th} day as shown in Fig-4, at this exposure level, the brain accumulates BMF~ 21X. As the exposure concentration increases the BMF is reduced. At the exposure level (17.33 ppm), the brain accumulated lesser amount of aluminium approximately 6X as shown in Fig-4. The uptake rate is low and elimination rate is high in the acute exposure (k_1=0.0448h^{-1} and k_2=0.0079h^{-1}) as shown in Fig-2, compared to chronic exposure (k1=0.0262 h^{-1} and k2=0.0013 h^{-1}) as shown in Fig-3. Low elimination rate in the chronic exposure results in maximum BMF (21X).

Accumulation of aluminium in the brain tissue of the fish exposed to acute and chronic treatment could be due to the disfunctioning of liver, the primary organ for detoxification of any toxicant. Also, in the brain, the blood-brain barrier effectively decreases the amount of toxic substances that are transferred to the brain tissues. The blood brain barrier is created by the close association of brain capillaries with specialized cell found in the nervous system. Hence, the considerable accumulation of found in brain tissue suggest that the toxic substance aluminium penetrated into the blood brain barrier and entered into the brain. In the brain, accumulation of metal in the organs of fish is a function of uptake and excretion rates. Uptake rate is considered the metal to the tissues and cell surfaces. The period of exposure has a considerable influence on the rate of accumulation of aluminium. The degree of interference depends on the concentration of the metal accumulated, which could differ not only among different animals but also amongst different organs of the same animals. So that it indicates that the metal uptake from water is the most important route of bioaccumulation [20].

Table-1: Accumulation, recovery, uptake, excretion rate and BMF of aluminium in the Brain and Liver tissues of *cirrhinus mrigala* fingerlings at acute and chronic exposures

Brain	Periods of exposure					
	Control	14 days	15 days	30 days	60 days	90 days
Al intoxicated	6.774	98.23	18.48	48.53	68.71	106.63
Al+DFO	6.774	36.89	9.35	12.49	19.86	38.31
Al+DFP	6.774	27.84	8.34	8.37	17.96	28.31
K1	6.774	0.0448	0.0099	0.0191	0.0213	0.00262
K2	6.774	0.0079	0.0028	0.0021	0.0016	0.0013
BMF	6.774	6X	4X	9X	9X	21X
Liver	Periods of exposure					
	Control	14 days	15 days	30 days	60 days	90 days
Al intoxicated	4.961	86.35	39.18	46.49	92.31	104.11
Al+DFO	4.961	52.21	33.89	38.25	52.75	62.01
Al+DFP	4.961	47.61	27.89	28.51	46.50	56.12
K1	4.961	0.0424	0.468	0.0208	0.0360	0.00282
K2	4.961	0.0085	0.0062	0.0023	0.0020	0.0014
BMF	4.961	5X	8X	9X	18X	20X

Liver

In the present investigation it was found that the liver tissues accumulated significant amount of aluminium in acute 86.35mg/g and chronic exposures 39.18mg/g, 46.49mg/g , 92.31mg/g and 104.11mg/g at 15, 30, 60 and 90 days respectively as shown in table-1. The treatment of the chelating agents DFO and DFP reduces the concentration of aluminium significantly 45% and 40% (acute), 14%-40% and 45% 46%(chronic) as shown in Fig-5. The accumulation and elimination of aluminium during acute chronic exposures are shown in the table-1. The uptake rate decreases suddenly during the initial period upto the 30^{th} day then after it remains almost constant upto the 90^{th}

day as shown in Fig-6. On the other hand the excretion rate decreases suddenly upto 30th day and decreases slowly afterwards as shown in Fig-7. Also the BMF increases slowly upto 30th day and increases suddenly upto 60th then after it increases slowly as shown in Fig-8.

In the present investigation, it could be seen from the Fig -8 that the greatest Biomagnifications factor was found at the lowest aluminium exposure level i.e. 5.2 ppm for 90 days, at this exposure level, the liver accumulated approximately 20X amount of aluminium present in the ambient medium. As the exposure concentration increases the BMF is reduced .At the highest exposure level (17.3 ppm), the liver accumulated the least amount of aluminium is 5X. Also the uptake rate is low and the elimination rate is high in acute exposure (k_1=0.042h^{-1} and k_2 =0.0085h^{-1}) compared to their rates in chronic exposure (k_1=0.0282h^{-1} and k_2=0.0014h^{-1}). The very low elimination rate during chronic exposures leads to maximum accumulation in the liver and consequently the highest BMF.

Liver plays an important role in contaminant storage, redistribution, detoxification or transformation and acts as an active site of pathological effects induced by contaminants. The metal binding protein metallothionein is of almost importance in the accumulation of metals. The liver is the main target organ for homeostasis in fish, for clearing the blood substance entering the circulation from the gastrointestinal tract passes through the liver before reaching the systematic circulation from the gastrointestinal tract passes through the liver before reaching the systematic circulation. Therefore, the liver remove the toxicants from the blood, biotransforms them into bile and presents their distribution to other parts of the body. Hence the liver, which is a major procedure of metal binding proteins, shows higher concentration of the heavy metals aluminium. The liver plays an important role in the detoxification process as metal elimination is routed through it and the liver is perhaps the last organ to be relieved of the aluminium metal load, this might possibly require a longer time for elimination.

In the present study liver receives least amount of aluminium for both acute and chronic exposures, the treatment of the chelating agent reduced aluminium concentration significantly (in the liver tissue) and the depletion goes down with increases in exposure and have reported most toxicant enters the body through the gastrointestinal track and after absorption they are carried to the liver and the accumulated [21]. Also the high doses of Al may reflect homeostatic process down regulating gene expression for pro-inflammatory elements by negative feedback [22]. Since the level of inflammatory markers was not changed in the serum (or) liver of treated animals following exposure to Al, the effects observed were not due to systemic changes, but rather reflect a selective vulnerability of nervous tissue.

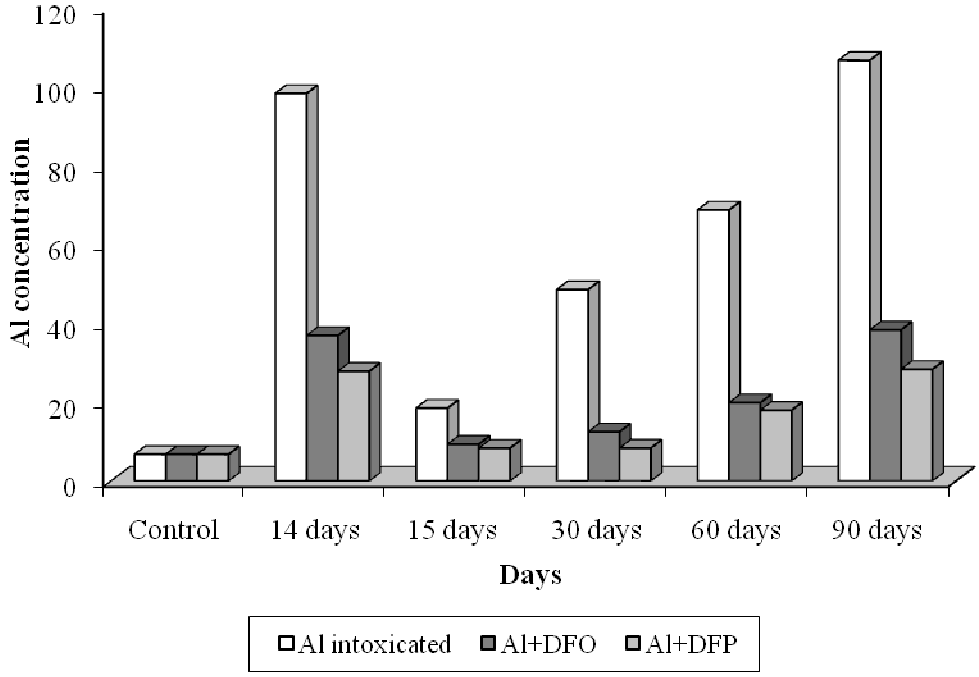

Fig. 1: Bioaccumualtion and elimination of Aluminium in Brain tissues during acute and chronic exposure

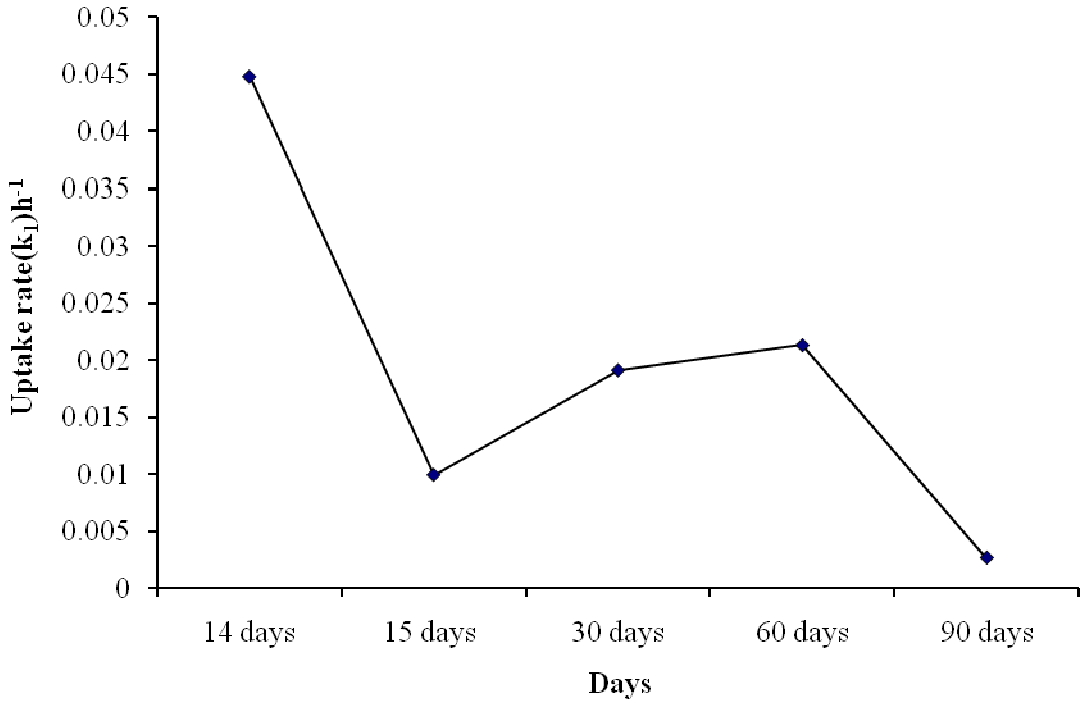

Fig. 2: Uptake rate (k_1) of Brain tissues during acute and chronic exposures

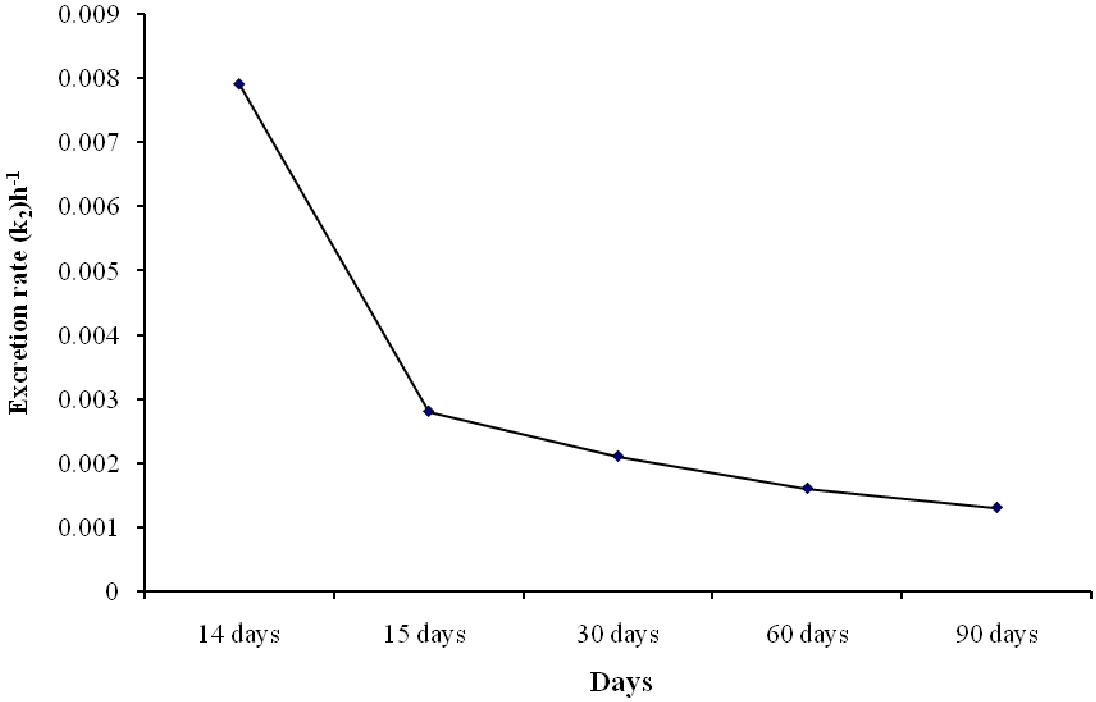

Fig. 3: Excretion rate (k_2) of Brain tissues during acute and chronic exposures

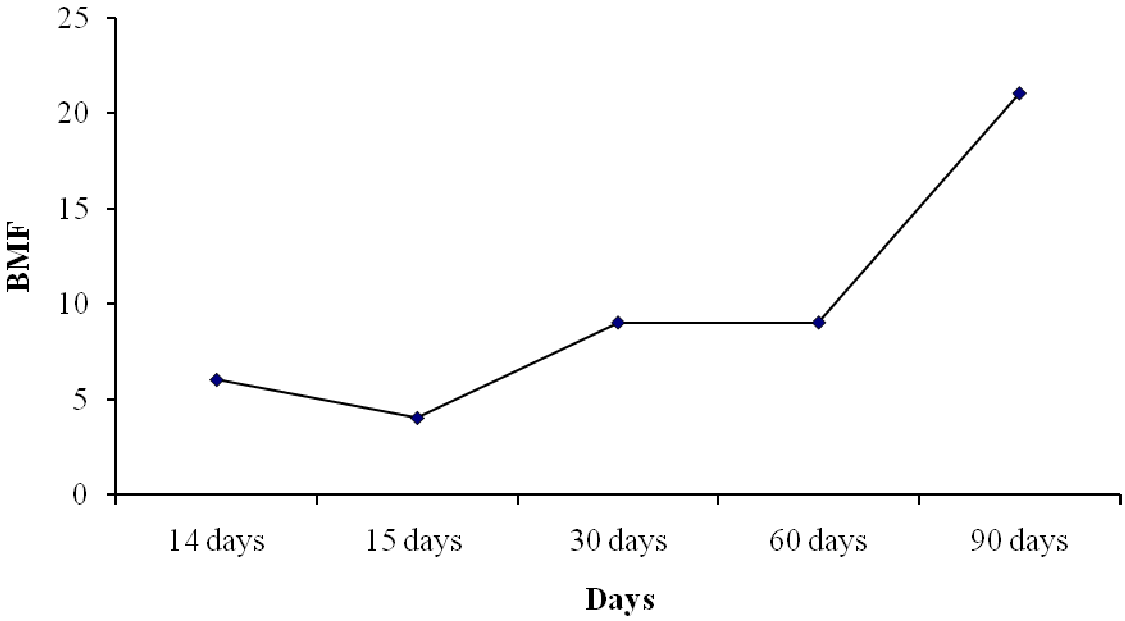

Fig. 4: BMF of Brain tissues during acute and chronic exposures

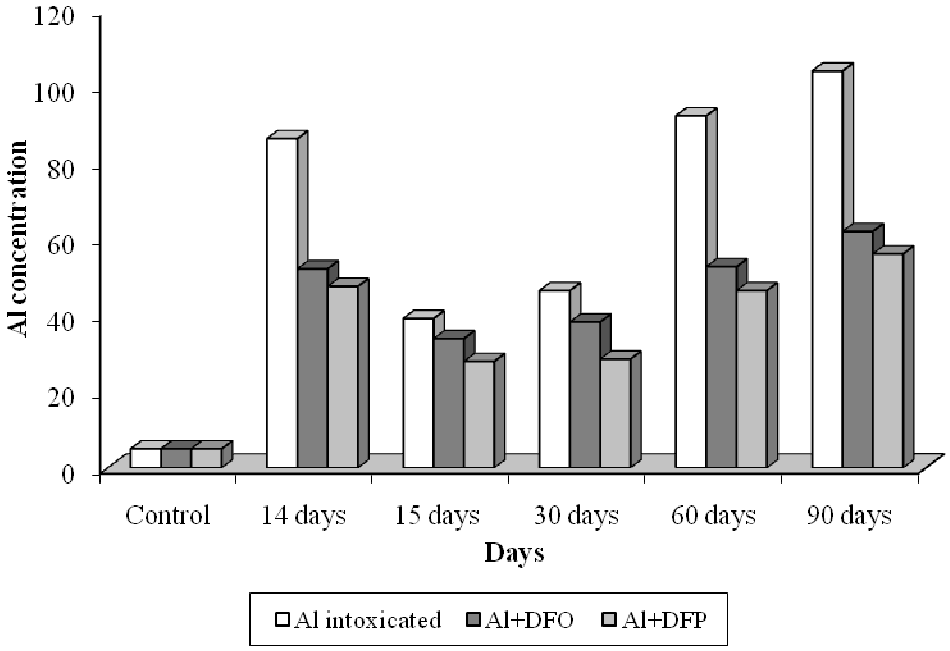

Fig. 5: Bioaccumualtion and elimination of Aluminium in Liver tissues during acute and chronic exposure

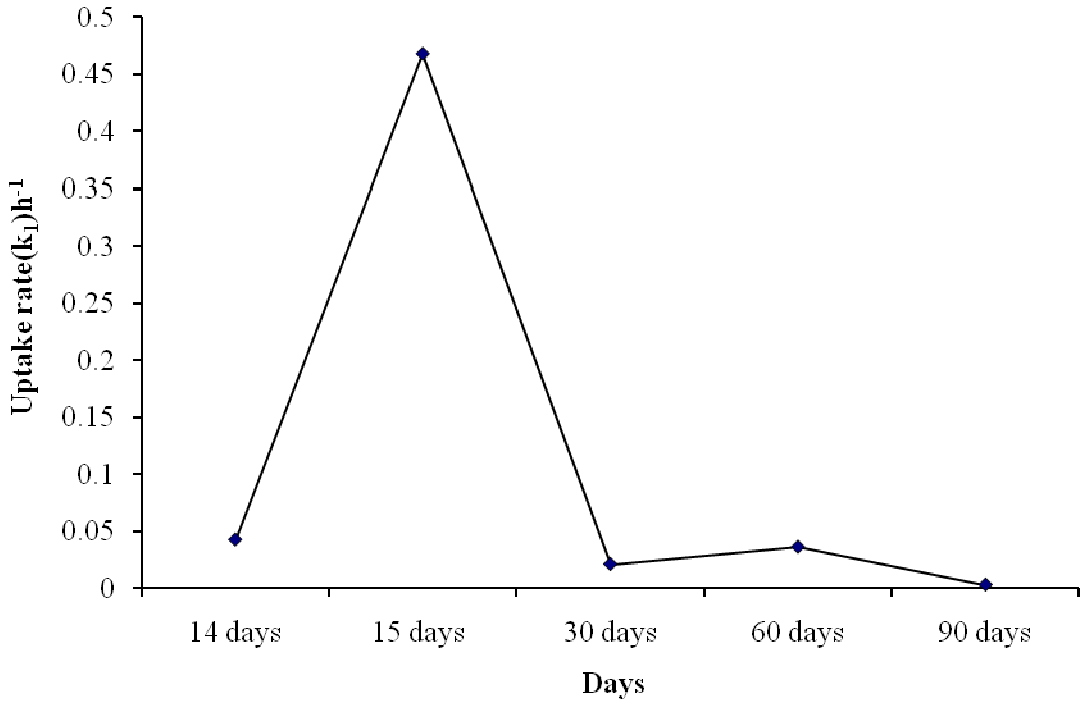

Fig. 6: Uptake rate (k_1) of Liver tissues during acute and chronic exposures

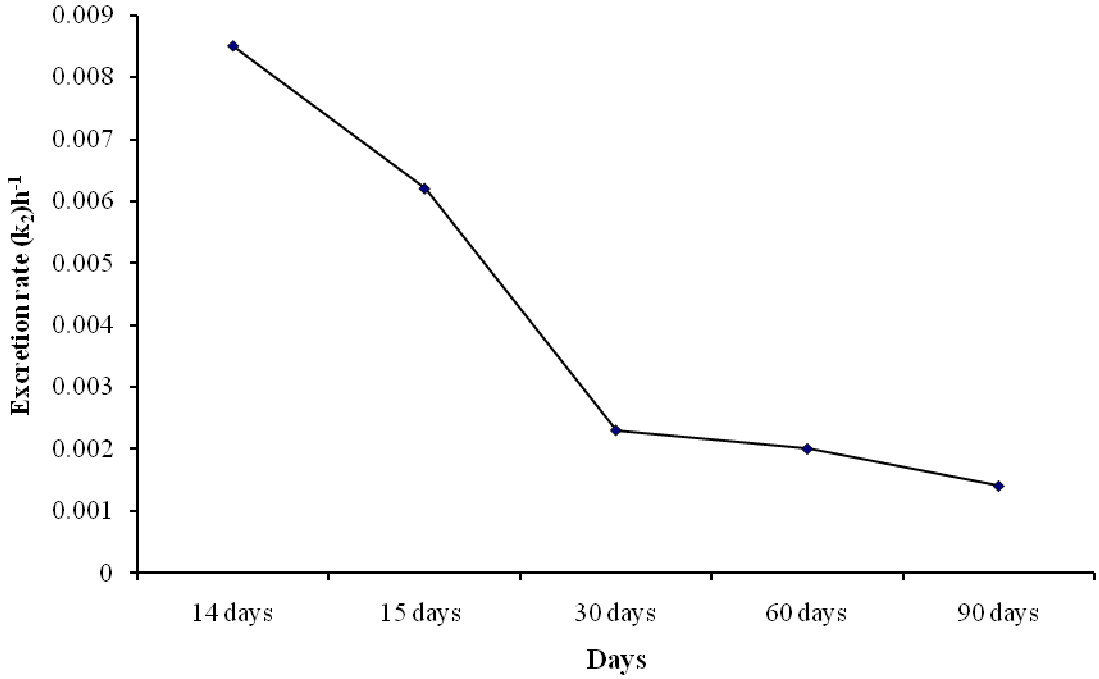

Fig. 7: Excretion rate (k_2) of Liver tissues during acute and chronic exposures

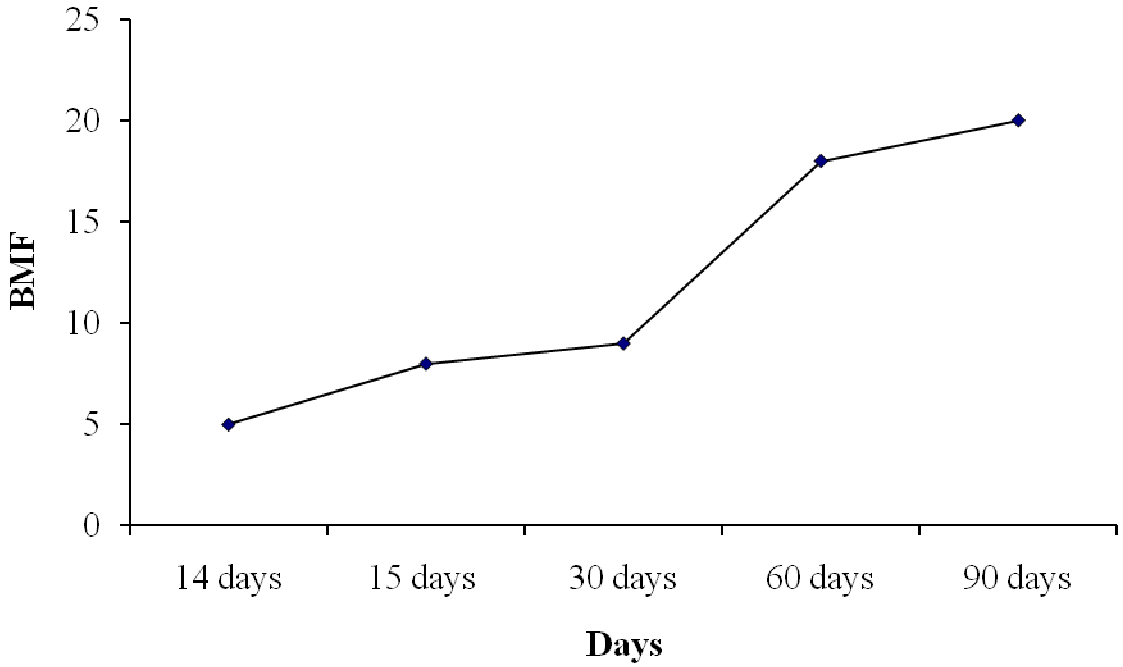

Fig. 8: BMF of Liver tissues during acute and chronic exposures

CONCLUSION

It has been found in the present study that the accumulation pattern follows the order: Brain>Liver by using ICP-AES. Chelating agents are most versatile and effective antidotes for metals intoxication and stable complexes, which can handily get accumulated in organisms, thereby reducing the toxicity of the metals to organisms. The present study suggests that in from ICP-AES is best instrument to find out the DFO and DFP reduced the aluminium concentration in the *Cirrhinus mrigala* fingerlings has found and the BMF is low for acute duration exposure.

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