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Aluminum and the effects of chelating agents on Muscle, Kidney and Liver 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 to eliminate the metals toxicities. The aim of our present study is to finds out bioaccumulations of aluminum and the effects of chelating agents DFO and DFP in Muscle, kidney and liver tissues of Cirrhinus mrigala by using Inductively Coupled Atomic Emission Spectrometer (ICP-AES). This study finds out the accumulation of aluminum is Muscle>Kidney>Liver. The present result suggests that DFO and DFP reduce the aluminum concentration in the tissues of Cirrhinus mrigala fingerlings and both are efficient chelator. Aluminum 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, aluminum, chelating agents, ICP-AES

INTRODUCTION

The estimation of aluminium concentration in the samples of fish had made using an ICP-AES (ISA JOBIN YVON 24 MODAL) and the analytical standard was 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 were 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

$$C = C_0 e^{-k2t}$$

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, Al_2 $(SO4)_3$, DFO and DFP were purchased from S.D. Fine, Novartis and Sigma, chemicals limited Mumbai, India.

Experimental design

Test specimens were divided into 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

Muscle

In the present study gave us Muscle tissues accumulates aluminum as 142.62 µg/g in acute exposure and 45.33 µg/g, 50.21 µg/g,73.22 µg/g and 159.25 µg/g in chronic exposure at 15, 30, 60 and 90 days respectively as shown in Table-1. Then treatment with chelating agents DFO and DFP reduced the concentration of aluminum in the muscle to significantly 73% and 71% for acute, 56%-71% and 50%-70% for chronic exposure respectively. Bioaccumulation and elimination of Aluminum in muscles during acute and chronic exposure as shown in Fig.1.The uptake rate (K_1) decreased upto 30 days and then gradually increased upto 90 days as shown in Fig.2, but the execration rate (K_2) decreased upto 30 days and slowly decreased up to 60 days and remain almost constant upto 90 days as shown in Fig.3. Also the BMF increased gradually upto 60 days and after increased upto 90 days as shown in Fig.4. The greatest bioconcentration factors occurred at the lowest exposure level 5.2 ppm for 90 days, at that exposure level muscle accumulated approximately 31X amount of aluminum. At the highest exposure level 17.33 ppm, the muscles accumulated the least amount of aluminum so that uptake rate was low and elimination rate was high in the acute exposure (K_1 =0.0756h^{-1} and K_2= 0.0092 h^{-1}) as shown in Table-1. When compared to chronic exposure the elimination rate was very low in chronic exposure as shown in Fig.3, and it remain constant after the 30 days, which gave in maximum BMF 31X as shown in Fig.4. Therefore, the present study gave us Fishes were known for their ability to concentrate heavy metals in their muscles and various organs, also substantial amount of aluminum was observed in muscle for chronic and acute exposures and the heavy metals mainly through the blood [20].

Table-1: Accumulation, recovery, uptake, excretion rate and BMF of aluminum in the muscles tissue of *Cirrhinus mrigala* at acute and chronic exposures

Muscle	Periods of exposure					
	Control	14 days	15 days	30 days	60 days	90 days
Al intoxicated	6.49	142.62	45.33	50.21	73.22	159.25
Al+ DFO	6.49	41.36	22.85	28.67	29.40	47.18
Al+ DFP	6.49	39.11	20.16	25.32	28.11	46.72
K1		0.0756	0.0469	0.0203	0.0289	0.046
K2		0.0092	0.0054	0.0021	0.0017	0.0015
BMF		8X	9X	10X	14X	31X

Values are statistically significant between the control and treated is p<0.05.

Corresponding plots are as shown below:

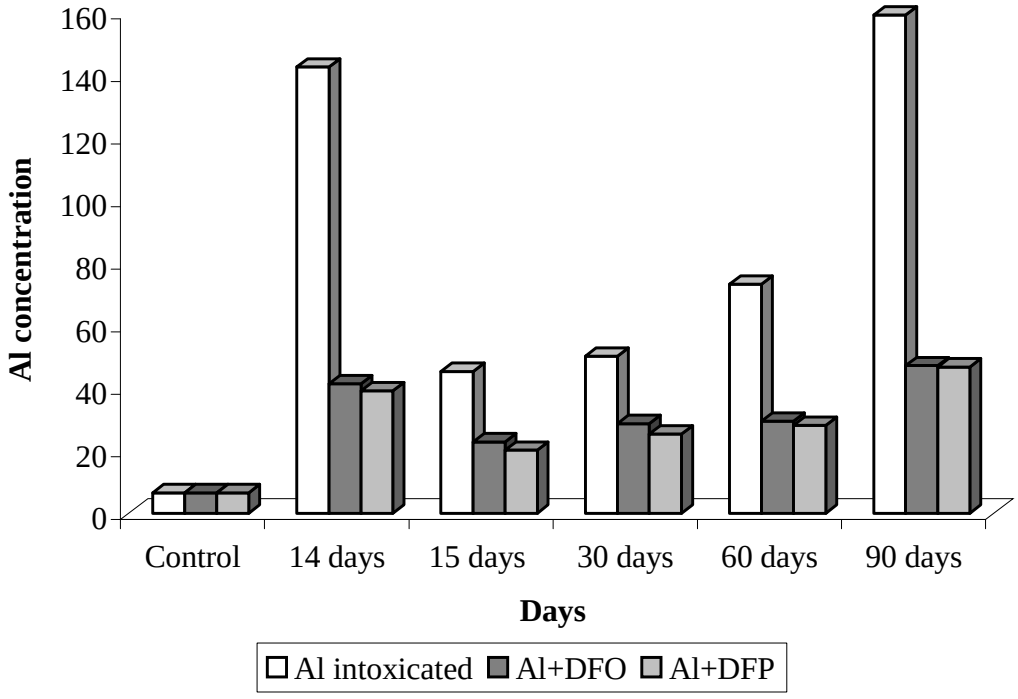

Fig. 1: Bioaccumualtion and elimination of Aluminium in muscle tissues during acute and chronic exposure

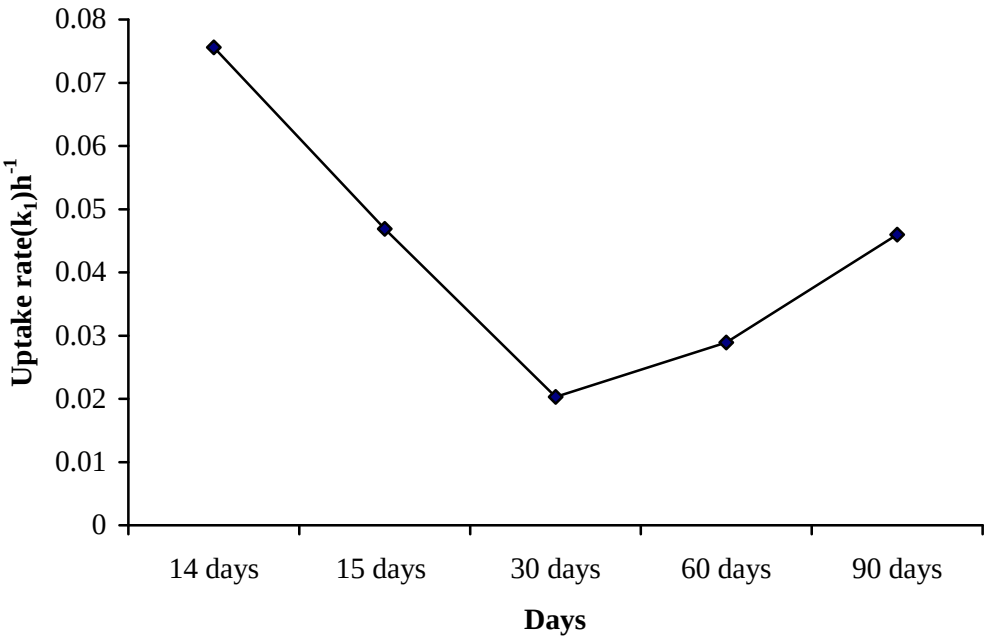

Fig. 2: Uptake rate (k_1) of muscle tissues during acute and chronic exposures

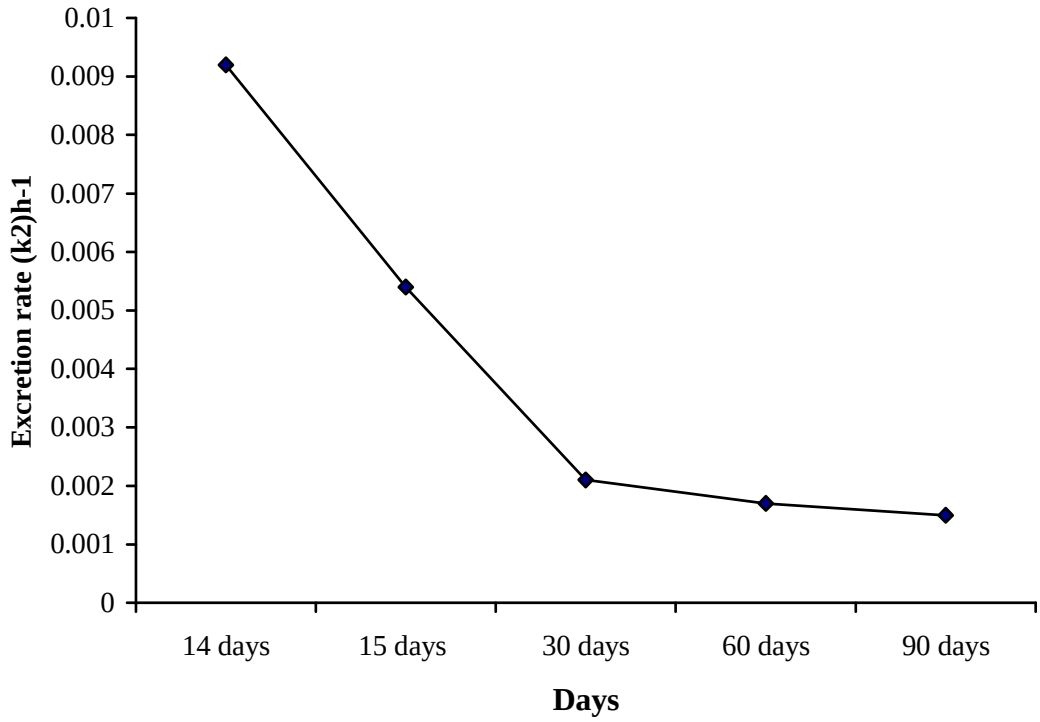

Fig. 3: Excretion rate (k_2) of muscle tissues during acute and chronic exposures

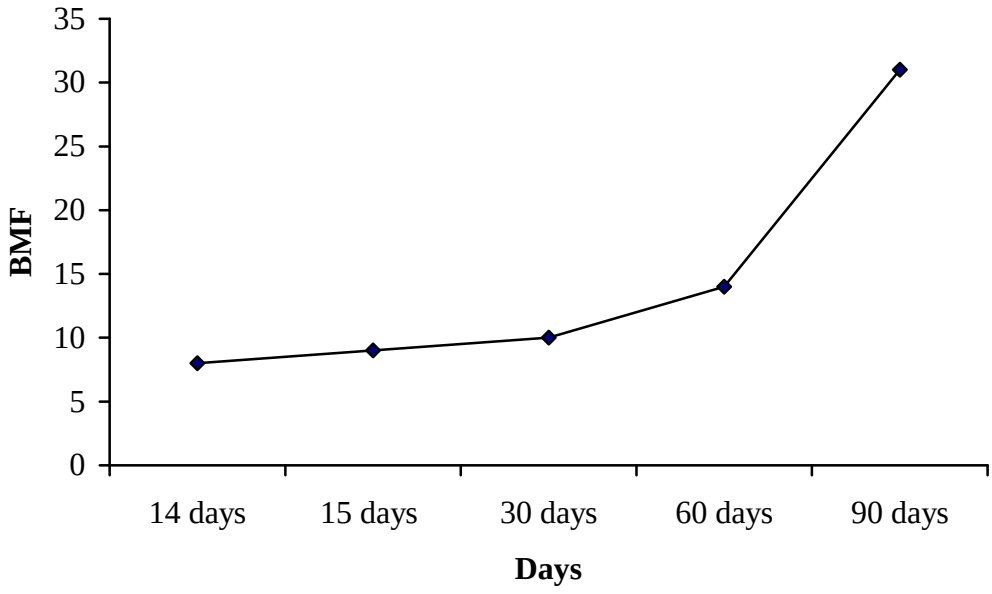

Fig. 4: BMF of muscle tissues during acute and chronic exposures

Kidney

Kidney tissues accumulated a higher amount of aluminum 106.52 µg/g for acute and for chronic 35.50 µg/g, 44.51 µg/g, 47.61 µg/g, 112.50 µg/g at15, 30, 60 and 90 days respectively as shown in Table-2. Treatment with the chelating agents DFO and DFP reduced the concentration of aluminum in the kidney was 60.28 µg/g for acute and for chronic 16.45 µg/g, 19.50 µg/g, 26.75 µg/g and 30.57 µg/g at 15,30,60 and 90 days respectively as shown in Fig.5. The accumulation and elimination of aluminum in acute and chronic exposure, the variation of the uptake rate and excretion rate were as shown in Fig.5, Fig.6 and Fig.7 respectively. The uptake rate decreased gradually upto 60 days and increased suddenly afterwards upto 90 days as shown in Fig.6, on the other hand the excretion rate was gradually decreased from 15 days to 90 days as shown in Fig.7. BMF values were increased slowly for 15 to 60 days

and afterwards increased rapidly as shown in Fig.8. It was inverse manner as that of excretion rate. From the comparison of uptake rate and BMF of kidney tissues, it was seen that the greatest biological magnification factor occurred at the chronic exposure for 90 days. At this exposure level, the kidney accumulated approximately 22X amount of aluminum. As the exposure concentration increased the BMF was reduced as shown in Fig.6. At the highest exposure level the kidney accumulated the least amount of aluminum approximately 6X. Also the uptake rate was low and elimination rate was high in the acute exposure K_1= 0.0563 h^{-1} and K_2= 0.0092 h^{-1} compared to the chronic exposure K_1= 0.0563 h^{-1} and K_2= 0.0015 h^{-1}as shown in Table-2. The very low elimination rate during the chronic exposures leads to maximum BMF was 22X. The treatment of the chelating agents DFO and DFP reduced the concentration of aluminum significantly 43% and 53% for acute, 61% to 73% and 54% to 74% for chronic as shown in Fig.5. The excretory organs usually accumulated large quantities of metals especially in the fish and animals. During the excretion process the excess amount of aluminum ions are rapidly eliminated from the body through the kidney mainly detoxification mechanism. Hence kidney is clearly a major target organ for both acute and chronic aluminum exposures [20]. Aluminum overload in renal patients causes a number of diseases such as Dementia, Alzheimer's disease, and memory loss [21].

Table-2: Accumulation, recovery, uptake, excretion rate and BMF of aluminum in the Kidney tissues of *Cirrhinus mrigala* at acute and chronic exposures

Kidney	Periods of exposure					
	Control	14 days	15 days	30 days	60 days	90 days
Al intoxicated	4.852	106.52	35.50	44.51	47.61	112.50
Al+ DFO	4.852	60.28	16.45	19.50	26.75	30.57
Al+ DFP	4.852	49.75	13.73	17.50	20.52	29.67
K1		0.0563	0.0378	0.0198	0.0145	0.0315
K2		0.0092	0.0055	0.0023	0.0016	0.0015
BMF		6X	7X	8X	9X	22X

Values are statistically significant between the control and treated is p<0.05.

Corresponding plots are as shown below:

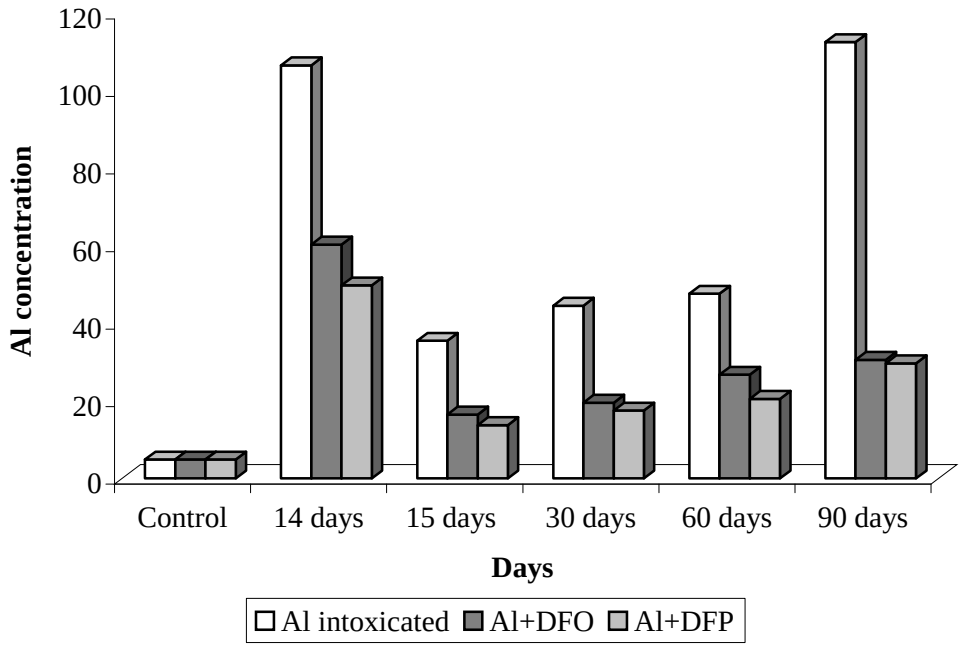

Fig. 5: Bioaccumualtion and elimination of Aluminium in Kidney tissues during acute and chronic exposure

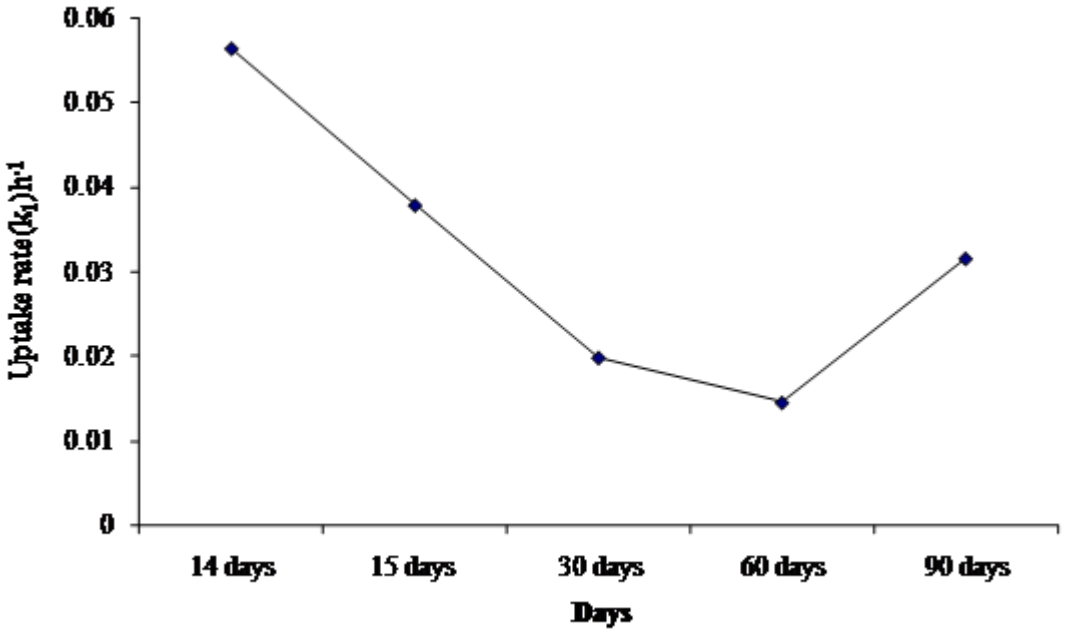

Fig. 6: Uptake rate (k_1) of Kidney tissues during acute and chronic exposures

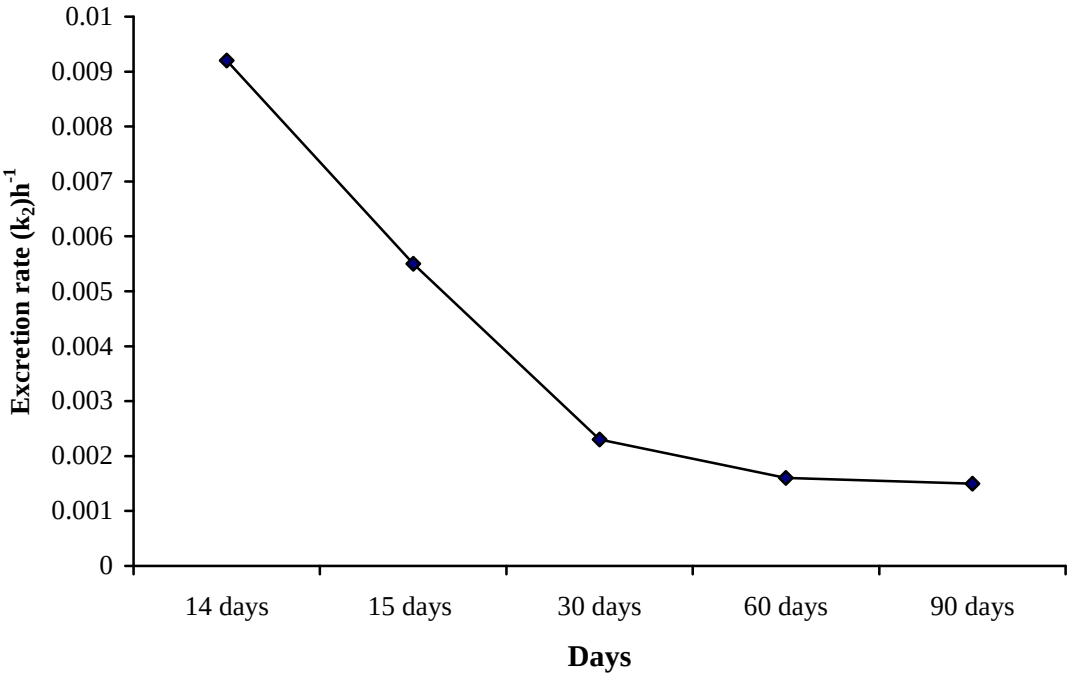

Fig. 7: Excretion rate (k_2) of Kidney tissues during acute and chronic exposures

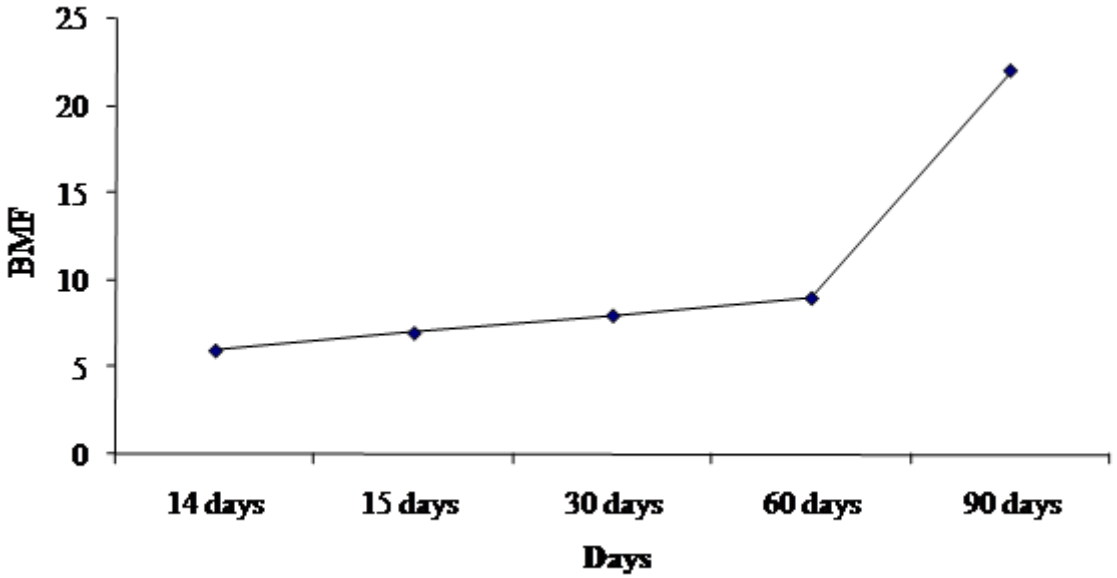

Fig. 8: BMF of Kidney tissues during acute and chronic exposures

Liver

In the present investigation found that the liver tissues accumulated significant amount of aluminum in acute 86.35µg/g and chronic exposures 39.18µg/g, 46.49µg/g, 92.31µg/g and 104.11µg/g at 15, 30, 60 and 90 days respectively as shown in Table-3. The treatment of the chelating agents DFO and DFP reduced the concentration of aluminum significantly 45% and 40% for acute, 14%-40% and 45% 46% for chronic exposure as shown in Fig.9. The accumulation and elimination of aluminum during acute chronic exposures were shown in the Fig.9. The uptake rate decreased suddenly during the initial period upto the 30 days then after it remain almost constant upto the 90 days as shown in Fig.10. On the other hand the excretion rate decreased suddenly upto 30 days and decreased slowly afterwards as shown in Fig.11. BMF increased slowly upto 60 days then after it increased slowly as shown in Fig.12.

In the present investigation, the greatest Biomagnifications factor was found at the lowest aluminum exposure level 5.2 ppm for 90 days, at this exposure level, the liver accumulated approximately 20X amount of aluminum. As the exposure concentration increased then BMF was reduced. At the highest exposure level 17.3 ppm, the liver accumulated the least amount of aluminum and its BMF was 5X. Also the uptake rate was low and the elimination rate was 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 of aluminum 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 aluminum. 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 aluminum metal load, this might possibly require a longer time for elimination.

In the present study liver received least amount of aluminum for both acute and chronic exposures, the treatment of the chelating agents reduced aluminum concentration significantly in the liver tissue and the depletion was down with increased in exposure and it reported most toxicant enters the body through the gastrointestinal track and after absorption they are carried to the liver and the accumulated [22]. Also the high doses of Al may reflect homeostatic process down regulating gene expression for pro-inflammatory elements by negative feedback [23]. 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.

Table-3: Accumulation, recovery, uptake, excretion rate and BMF of aluminum in the Liver tissues of *Cirrhinus mrigala* at acute and chronic exposures

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		0.0424	0.468	0.0208	0.0360	0.00282
K2		0.0085	0.0062	0.0023	0.0020	0.0014
BMF		5X	8X	9X	18X	20X

Values are statistically significant between the control and treated is p<0.05.

Corresponding plots are as shown below:

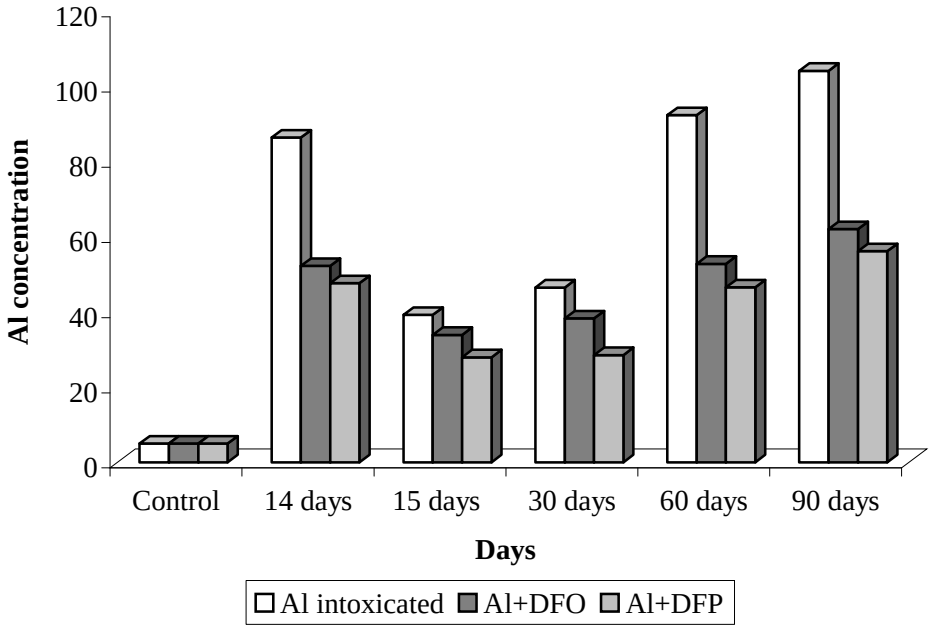

Fig. 9: Bioaccumulation and elimination of Aluminum in Liver tissues during acute and chronic exposure

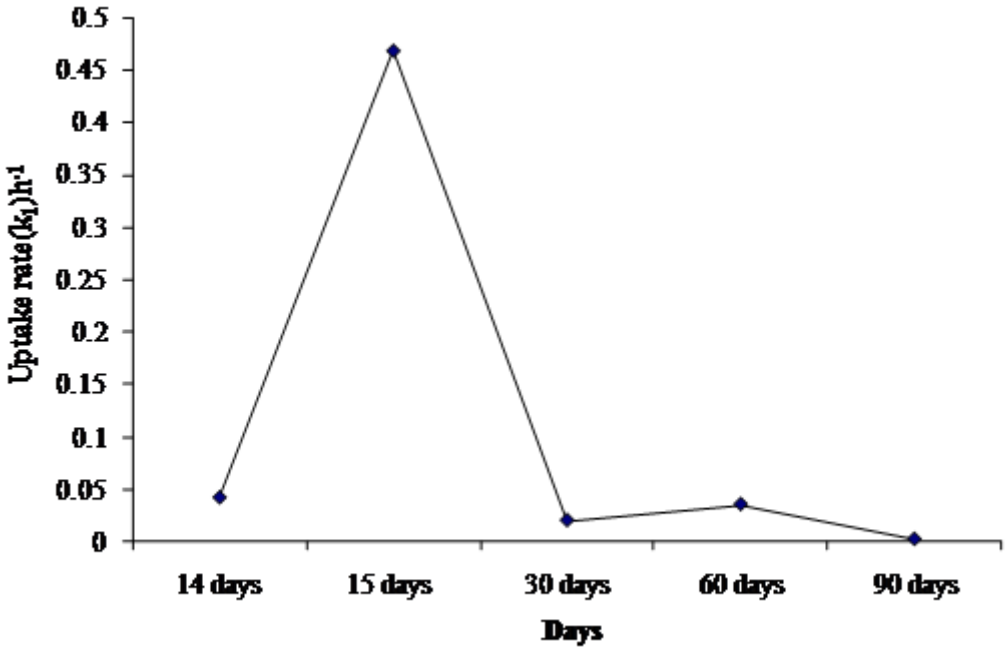

Fig. 10: Uptake rate (k_1) of Liver tissues during acute and chronic exposures

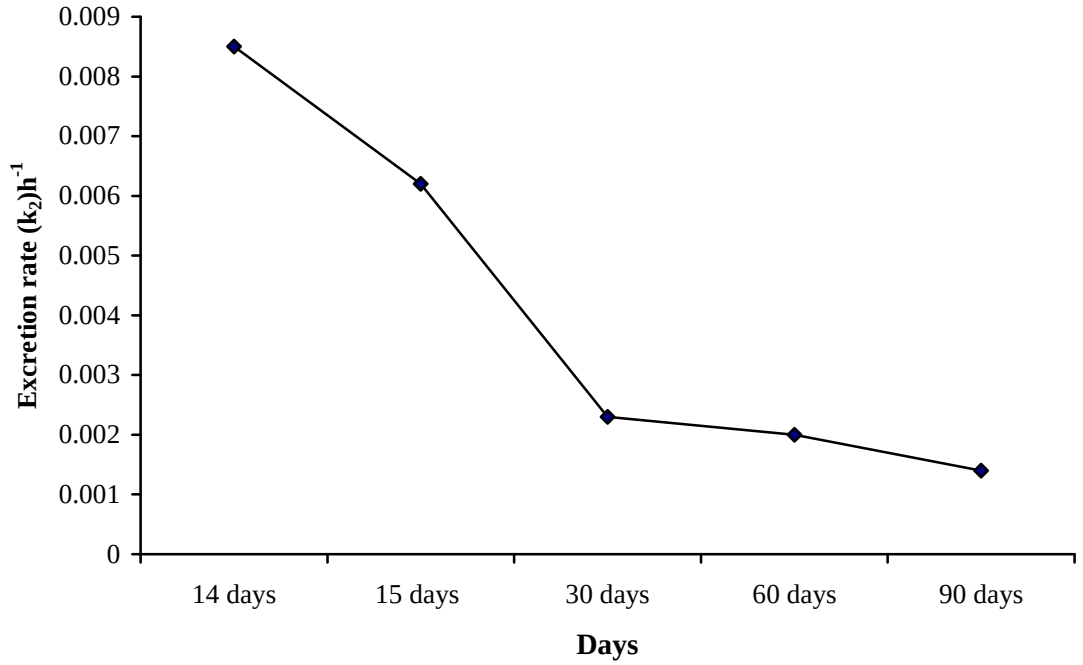

Fig. 11: Excretion rate (k_2) of Liver tissues during acute and chronic exposures

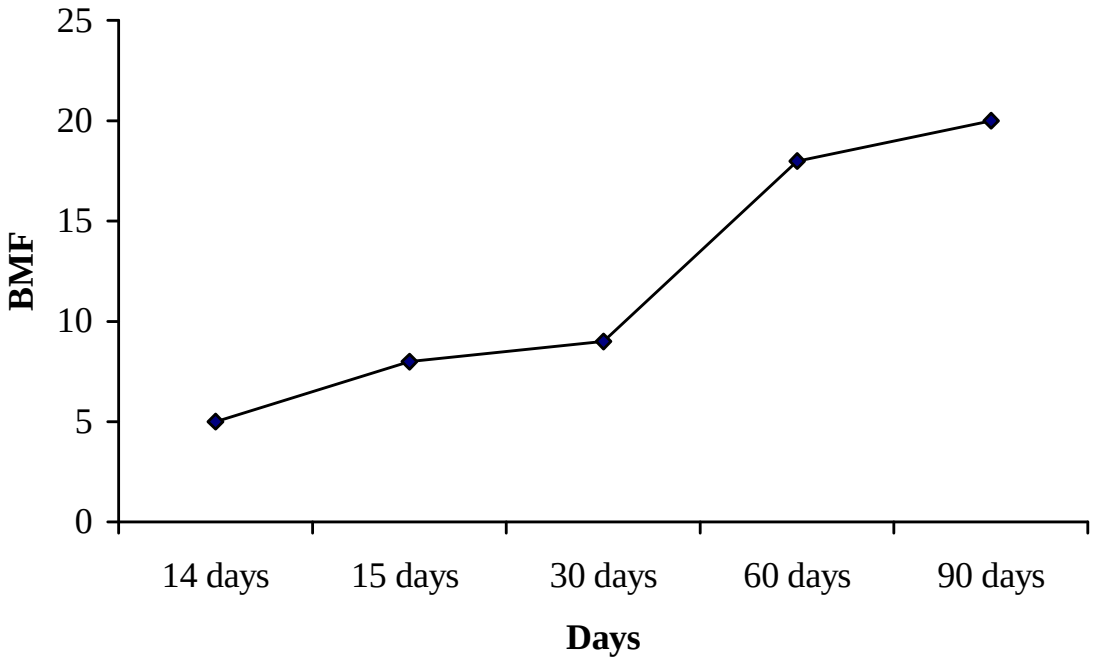

Fig. 12: BMF of Liver tissues during acute and chronic exposures

CONCLUSION

It had been found in the present study that the accumulation pattern follows the order: Muscle>Kidney>Liver by using ICP-AES. Chelating agents were 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 was to find out the DFO and DFP reduced the aluminum concentration in the *Cirrhinus mrigala* fingerlings and the BMF was low for acute exposure period.

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