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Research Article

TOXICOKINETICS PATTERN OF FLUORIDE IN CAT FISH, *CLARIAS BATRACHUS*

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ABSTRACT. One of the major ground water pollutants is Fluoride, which is contributed in the environment by both natural (geogenic) and anthropogenic means. Fluorine is an essential element for living organism. Chronic exposure study of 60 days was carried out in model fish, *Clarias batrachus*, where the fishes were divided into three groups; control (Group I), 10 mgL^{-1} (Group II) and 20mgL^{-1}(Group III) NaF.In a regular interval period of 15 days, accumulation status were reported in biological tissues. Maximum fluoride accumulation was found in Bone followed by liver, kidney, muscle and blood. After exposure of fishes to NaF for 60 days, depuration pattern was studied in group II and group III. Fishes from exposed groups were transferred to clean water, for a period of 20 days. Maximum depuration was observed in liver tissues followed by muscle, bone, kidney, blood. Depuration in group III was in the order; liver followed by kidney and muscle, bone and blood.

Keywords: *Chronic exposure, Cat fish, fluoride, accumulation, depuration.*

INTRODUCTION

One of the major ground water pollutants is fluoride, which is contributed in the environment by both natural (geogenic) and anthropogenic means. Fluorine is an essential element for living organism. The inorganic form, fluoride is essential for the development of bones and teeth. Fluoride when administered more than its reference value, can cause fluorosis. Fluorosis can be of skeletal or non-skeletal types. The permissible level of fluoride in drinking water is 1.5gL^{-1}[1].

Directly from water and through diet, are two ways aquatic animals acquire fluoride in their system. Accumulation of fluoride via food chain is very common and significant in case of fishes [2]. This accumulated fluoride is either distributed and stored in different body parts or is eliminated out of the body via renal excretory system. According to Camargo [3] some invertebrates and fishes on acute exposure to fluoride in contaminated water, accumulate high concentrations in their body. The exoskeleton of invertebrate animals tends to aggregate more fluoride than muscular system [4]. This plays an important role in hardening of exoskeleton where higher concentration of inorganic

fluoride binds with phosphorus and calcium forming $Ca_5(PO_4)_3F$ which functions as hardener of invertebrate exoskeleton [5]. Along with storage of fluoride in exoskeleton, soft tissues of some invertebrates have capability to store fluoride in them with existence of linear relationship between concentrations of fluoride in water to fluoride stored in tissues [3].

It is also established and proved that there is a positive relationship between an increased water temperature and increased fluoride accumulation by invertebrates, possibly due to an increase in metabolism. Soft tissues, cartilages and bones are prime site of accumulation of ingested fluoride in fish species [2]. Muscles have been reported to accumulate less fluoride in comparison to bones. The Nature of transport of fluoride in skeletal bones and other calcified structures is of active type because it is a second order reaction [6]. Deleterious effects of fluoride in these animals are chiefly exerted on calcified skeletal structures, GI tract, kidneys, liver, neural organs, endocrine system and reproductive system [7]. The present study aims at determining the relationship between sodium fluoride exposure and its accumulation in different tissues. Depuration rates in different tissues were also estimated after the exposure periods of 60 days.

MATERIALS AND METHODS

Acclimatization of model fishes

The model organism, *Clarias batrachus* were purchased from the local market. They were examined for any wounds, skin lesions, or physical abnormalities before being selected for the experiment. Selected fish were placed in glass aquariums with a capacity of 50 litres and allowed to acclimatize for 15 days. Fishes were dipped in 0.1% KMnO4 for 1-2 minutes to prevent disease, infection, or injury before acclimatization. To give fish a clean and healthy habitat during acclimatization, the water was changed every 24 hours. Water used for acclimatization was regular tap water, with pH of 6.8. They were kept in an animal home with light and a temperature of 27 °C. Fishes were provided with proper oxygen supply and were fed twice daily. Acclimatization process for all the fishes (control and experimental) were done in a similar manner.

Exposure to toxicant (NaF)

After the completion of acclimatization period, fishes were divided into three categories; one control (Group I) and two experimental groups (Group II and III). All the fishes were exposed to sodium fluoride for a period of 60 days (Chronic exposure). No toxicant was added to group I, they were kept in normal tap water. Group II and III were exposed to 10 mgL^{-1} and 20mgL^{-1} NaF respectively.

Concentrations for chronic exposure of toxicants were decided considering the LC_{50} values of NaF. LC_{50} value of NaF for 96 hrs was 619.3 mgL^{-1}[8]. So, for long term exposure, below $1/10^{th}$ values of LC_{50} were taken for experiments.

Fishes from each group were sacrificed in a time interval of 15 days, for collection of biological samples such as blood, liver, kidney, muscles and bones. Accumulation status of NaF in various biological samples was estimated on 15^{th}, 30^{th}, 45^{th}, and 60^{th} day.

Quantification of fluoride

Quantitative analysis was done in the laboratory with the help of Thermo Fisher Scientific Orion 9609 BNWP ion selective fluoride electrode [9]. Water Fluoride analysis was done with the help of direct determination method.

Blood fluoride concentration fluoride was estimated with the help of analyte addition method by adding TISAB in all samples, using the formula shown in Eqn.1;

$$\textbf{Eqn. 1} \quad C_U = C_S[(V_U / V_S + V_U) \times 10^{\Delta E/S}]$$

Where, C_U = concentration of unknown sample, C_S= concentration of standard sample, VU = volume of unknown sample, V_S = volume of standard sample, $\Delta E = E_2 - E_1$, is the change in the electrode potential after addition, E_2 = mV after addition of sample, E_1=mV before addition of sample, S = slope of the electrode.

Fluoride in tissue samples were estimated by direct determination method. They were digested prior to analysis by the method of Birkeland [10]. Tissues collected after dissecting fishes at intervals of 15 days, were dried at 105°C in a closed compartment for about 24 hrs. 200 mg of dried and homogenized tissue sample was taken and was allowed to dissolve in 2ml of mixture of 11.6M perchloric acid and 14.3M nitric acid in 1:1 ratio. This mixture was then neutralized to a pH of 5.5 with the help of citrate buffer. Citrate buffer was prepared using equal volumes of 7.8M sodium hydroxide and 1.0M trisodium citrate. The prepared sample was used for fluoride analysis.

Depuration

The fishes were then subjected to study the depuration pattern of NaF in different tissues. Fishes from the experimental groups were transferred into tanks containing pure and clean water for purging of chemicals and contaminants. Fishes from every group were sacrificed over a time interval of 5 days (5^{th}, 10^{th}, 15^{th} and 20^{th} day) to obtain depuration status in various biological tissues.

Two way ANOVA, involving 2 variables, dose and duration and Duncan's multiple range test were applied to the data of the present study using SPSS 22 version biostatistical tool.

RESULTS AND DISCUSSION

Accumulation

Table 1 depicts amounts of fluoride accumulated in the experimental fish, *Clarias batrachus* of control and groups exposed to NaF. Fluoride was measured in biological samples like blood, liver, kidney, muscles and bone. Levels of fluoride in blood, liver, kidney, muscles and bone samples of *Clarias batrachus,* were measured at a regular interval of 15 days. Highest fluoride concentration was estimated in bone samples (2.017 ± 0.045 mgL^{-1}) of group III on 60^{th} day. Lowest concentration was found in blood samples ($0.6 \pm 3.5 * 10^{-5}$) of control group. Decreasing order of fluoride accumulation was found to be Bone > Liver > Kidney > Muscle > Blood.

Linear regression analysis was also calculated in all the biological tissues with respect to water fluoride concentration present in the fish tanks. Linear regression of fluoride levels in soft tissues and bone tissue on 15^{th} day is presented in Figure 1 and 2. Similarly,

for 30th, 45th and 60th day, fluoride levels are presented in Figure 3 and 4, Figure 5 and 6 and Figure 7 and 8 respectively.

Table 1. *Fluoride levels in blood (μgL^{-1}) and tissue samples (in mgL^{-1}) exposed to different concentrations of sodium fluoride for 60 days. Mean±SE (n=3); Group I-Control, Group II-10mgL^{-1}F, Group III-20mgL^{-1}F*

Tissue sample	Exposure duration (days)	Experimental groups Group I	Group II	Group III
Blood	15	$0.60\pm3.5*10^{-5}$	9.14±0.00019	11.10±0.0008
	30	$0.68\pm3.9*10^{-5}$	10.42±0.000279	11.59±0.0002
	45	$0.36\pm9.1*10^{-5}$	11.48±0.000173	12.54±0.0001
	60	$0.29\pm3.2*10^{-5}$	$12.27\pm3.4*10^{-5}$	$13.06\pm4.3*10^{-5}$
Kidney	15	$0.018\pm3.8*10^{-4}$	$0.038\pm6.5*10^{-4}$	$0.042\pm3.3*10^{-4}$
	30	$0.022\pm2.2*10^{-4}$	$0.045\pm1.7*10^{-3}$	$0.053\pm6.1*10^{-4}$
	45	$0.018\pm9.4*10^{-5}$	$0.054\pm8.2*10^{-4}$	$0.054\pm2.03*10^{-4}$
	60	$0.023\pm7.2*10^{-4}$	$0.059\pm1.1*10^{-4}$	$0.064\pm2.1*10^{-4}$
Liver	15	$0.018\pm4.4*10^{-4}$	$0.045\pm3.7*10^{-3}$	$0.045\pm6.37*10^{-4}$
	30	$0.022\pm2.6*10^{-4}$	$0.048\pm1.05*10^{-3}$	$0.054\pm8.4*10^{-5}$
	45	$0.020\pm3.6*10^{-5}$	$0.056\pm1.7*10^{-4}$	$0.062\pm1.05*10^{-3}$
	60	$0.019\pm2.5*10^{-4}$	$0.064\pm1.7*10^{-4}$	$0.069\pm1.48*10^{-4}$
Muscle	15	$0.018\pm1.27*10^{-4}$	$0.037\pm8.58*10^{-4}$	$0.040\pm6.5*10^{-5}$
	30	$0.019\pm9.4*10^{-5}$	$0.044\pm2.06*10^{-5}$	$0.048\pm3.36*10^{-5}$
	45	$0.017\pm7.05*10^{-5}$	$0.052\pm1.35*10^{-4}$	$0.055\pm3.81*10^{-4}$
	60	$0.019\pm1.3*10^{-4}$	$0.061\pm1.22*10^{-4}$	$0.064\pm1.63*10^{-4}$
Bone	15	1.12±0.071	1.42±0.012	1.58±0.027
	30	1.26±0.031	1.53±0.006	1.66±0.005
	45	1.43±0.043	1.64±0.006	1.82±0.030
	60	1.26±0.006	1.83±0.047	2.02±0.045

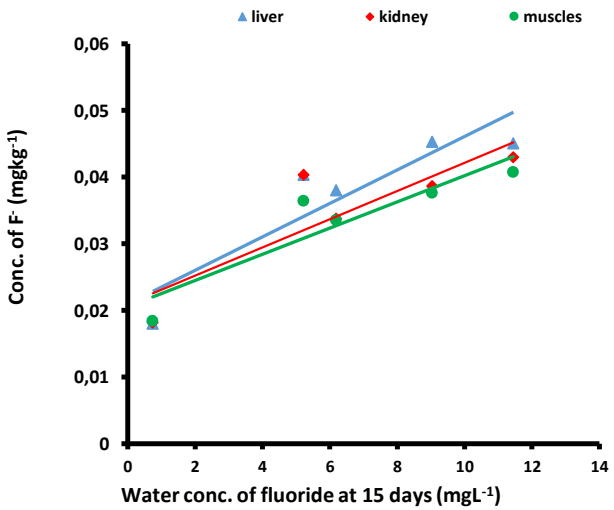

Fig.1. *Linear regression of fluoride levels in different soft tissues from Clarias batrachus in relation to fluoride level in exposure medium (water) on 15th day. For liver- (r = 0.907, p = 0.03), kidney- (r = 0.869, p=0.05), muscles- (r = 0.913, p =0.03).*

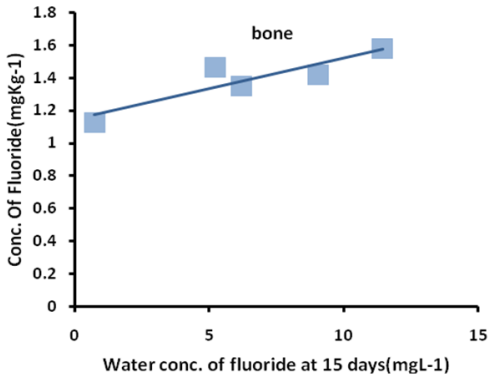

Fig.2. *Linear regression of fluoride levels in bone from Clarias batrachus in relation to fluoride level in exposure medium (water) on 15th day (r = 0.897, p = 0.038).*

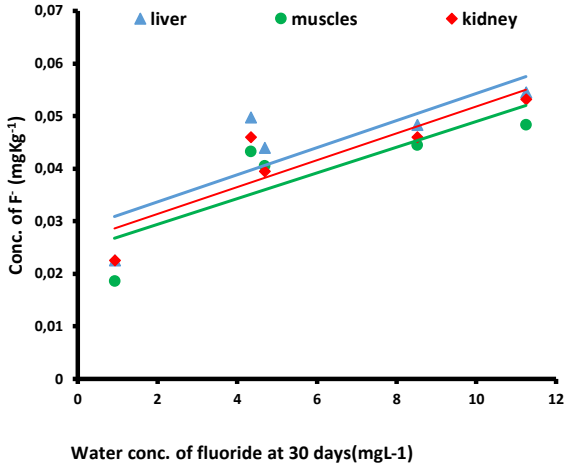

Fig.3. *Linear regression of fluoride levels in different soft tissues from Clarias batrachus in relation to fluoride level in exposure medium (water) on 30th day. For liver- (r = 0.828, p = 0.08), kidney- (r = 0.88, p=0.04), muscles- (r = 0.83, p =0.07).*

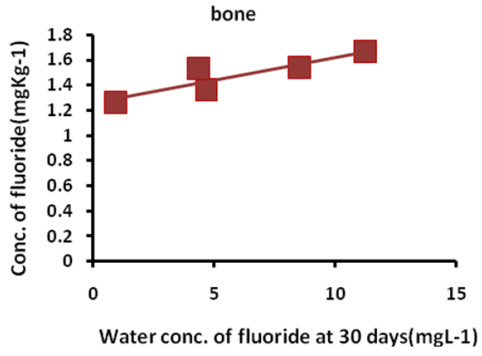

Fig.4. *Linear regression of fluoride levels in bone from Clarias batrachus in relation to fluoride level in exposure medium (water) on 30th day (r = 0.89, p = 0.04).*

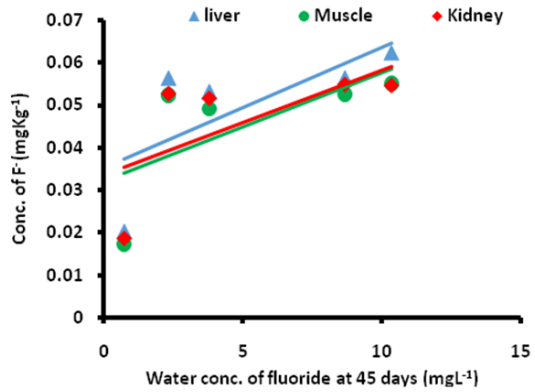

Fig.5. *Linear regression of fluoride levels in different soft tissues from Clarias batrachus in relation to fluoride level in exposure medium (water) on 45th day. For liver- (r = 0.702, p = 0.18), kidney- (r = 0.657, p=0.22), muscles- (r = 0.67, p =0.21).*

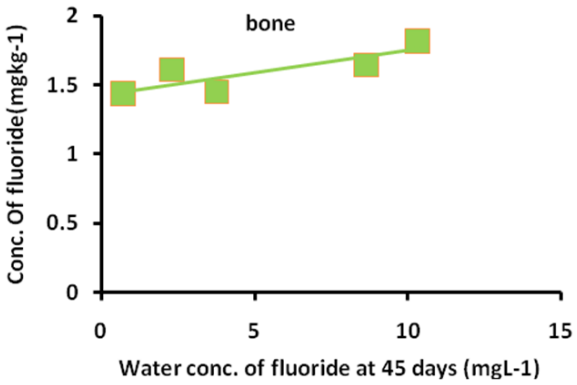

Fig.6. *Linear regression of fluoride levels in bone from Clarias batrachus in relation to fluoride level in exposure medium (water) on 45th day (r = 0.840, p = 0.07).*

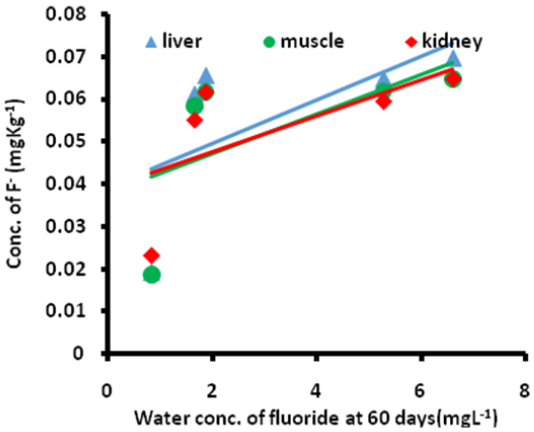

Fig.7. *Linear regression of fluoride levels in different soft tissues from Clarias batrachus in relation to fluoride level in exposure medium (water) on 60th day. For liver- (r = 0.622, p = 0.26), kidney- (r = 0.639, p=0.24), muscles- (r = 0.612, p =0.27).*

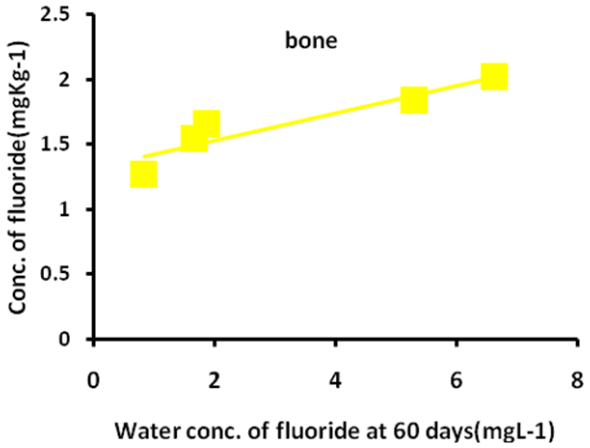

Fig.8. *Linear regression of fluoride levels in bone from Clarias batrachus in relation to fluoride level in exposure medium (water) on 60th day (r = 0.931, p = 0.02).*

Depuration

Periods of depuration projected for fluoride from blood and tissues of *Clarias batrachus*, exposed to different concentrations of fluoride (group II and III) are represented in table 2 and 3. After exposure of the desired NaF for 60 days, the experimental fishes were transferred to chemical-free, pure and clean water, for purging of chemicals or contaminants. Water sample taken for depuration studies was normal tap water (pH 6.8), similar to what we used in acclimatization period and also used for control samples. Depuration was carried out for 20 days. Maximum of 100 percent depuration of fluoride was observed in liver samples of group II and almost around 90 percent in the other group's fishes. Minimum amount of fluoride was depurated in blood samples. The order of depuration in group II was Liver (100%) > Muscle=Bone > Kidney > Blood (78.3%). Depuration in group III is in the order was Liver (99.9%) > Kidney = Muscle > Bone > Blood (72%).

Table 2. *Depuration pattern of fluoride from different tissues of Clarias batrachus of Group II (10mgL^{-1}F^{-}), after transfer to chemical free water at various time intervals*

Sample	**Depuration of Fluoride**		**Depuration rate**	**Significance level (Between the groups)**
	Depuration Period (Days)	Level Of Fluoride (mgL^{-1})	% change over control	F - value
Blood	0	0.012±5.1*10^{-5a}		3304.995
	5	0.009±6.1*10^{-5b}	16.1	
	10	0.006±8.6*10^{-5c}	52.2	
	15	0.004±2.4*10^{-5d}	69.6	
	20	0.003±5.8*10^{-5e}	78.3	

Table 2. *Continues*

Sample	Depuration of Fluoride		Depuration rate	Significance level (Between the groups)
	Depuration Period (Days)	Level Of Fluoride (mgL^{-1})	% change over control	F - value
Liver	0	0.065 ± 0.0001^{a}		207.272
	5	0.043 ± 0.0009^{b}	48.9	
	10	0.040 ± 0.0006^{b}	55.6	
	15	0.032 ± 0.0006^{c}	73.4	
	20	0.020 ± 0.0004^{d}	100	
Kidney	0	0.059 ± 0.0001^{a}		756.273
	5	0.046 ± 0.0005^{b}	33.4	
	10	0.036 ± 0.0006^{c}	59.0	
	15	0.031 ± 0.0003^{d}	71.8	
	20	0.024 ± 0.0005^{e}	89.8	
Muscles	0	0.061 ± 0.0001^{a}		1750.103
	5	0.049 ± 0.0005^{b}	27.9	
	10	0.039 ± 0.0001^{c}	51.2	
	15	0.028 ± 0.0006^{d}	76.8	
	20	0.020 ± 0.0001^{e}	99.9	
Bone	0	1.83 ± 0.047^{a}		69.947
	5	1.70 ± 0.003^{b}	23.3	
	10	1.66 ± 0.016^{b}	30.4	
	15	1.47 ± 0.024^{c}	64.3	
	20	1.29 ± 0.004^{d}	99.9	

Table 3. *Depuration pattern of fluoride from different tissues of Clarias batrachus of Group III ($20mgL^{-1}F^{-}$), after transfer to chemical free water at various time intervals*

Sample	Depuration of Fluoride		Depuration rate	Significance level (Between the groups)
	Depuration Period (Days)	Level Of Fluoride (mgL^{-1} and $mgKg^{-1}$)	% change over control	F - value
Blood	0	$0.013\pm4.3*10^{-5a}$		3883.044
	5	$0.010\pm5.6*10^{-5b}$	24.0	
	10	$0.007\pm2.7*10^{-5c}$	48.0	
	15	$0.005\pm8.2*10^{-5d}$	64.0	
	20	$0.004\pm7.4*10^{-5e}$	72.0	

Table 3. *Continues*

Sample	Depuration of Fluoride		Depuration rate	Significance level (Between the groups)
	Depuration Period (Days)	Level Of Fluoride (mgL^{-1} and $mgKg^{-1}$)	% change over control	F - value
Liver	0	0.069 ± 0.0001^{a}		984.148
	5	0.047 ± 0.0005^{b}	44.9	
	10	0.042 ± 0.0003^{c}	55.2	
	15	0.036 ± 0.0004^{d}	67.4	
	20	0.023 ± 0.0008^{e}	99.9	
Kidney	0	0.064 ± 0.0002^{a}		1677.237
	5	0.053 ± 0.0005^{b}	25.0	
	10	0.040 ± 0.0004^{c}	54.6	
	15	0.031 ± 0.0003^{d}	75.0	
	20	0.026 ± 0.0002^{e}	99.8	
Muscles	0	0.064 ± 0.0001^{a}		462.573
	5	0.051 ± 0.0006^{b}	28.3	
	10	0.043 ± 0.001^{c}	45.7	
	15	0.032 ± 0.0007^{d}	69.6	
	20	0.024 ± 0.0007^{e}	99.8	
Bone	0	2.01 ± 0.045^{a}		112.063
	5	1.86 ± 0.013^{b}	20.3	
	10	1.71 ± 0.007^{c}	40.6	
	15	1.60 ± 0.004^{d}	55.5	
	20	1.42 ± 0.006^{e}	79.8	

Fluoride tends to deposit maximum in bones as it get trapped in bones of the organism and has high affinity for calcium [11]. Accumulation can be followed by muscles, skin, liver or kidney. Fishes serve a major factor for fluoride accumulation in humans, through diet. Excess of fluoride can have serious implication on fish consuming population [12].

A positive correlation was established between concentration of fluoride in fishes and in exposure medium, in a study [13]. Another study, coinciding with our results; where sturgeon (*Acipenser baerii*) were exposed to different concentrations of 4, 10, 25, 62.5 mg L^{-1} NaF, showed maximum accumulation of fluoride concentration in bones (3204.4 mg kg(-1) dry weight. However, a smaller amount was detected in skin 100.1 mg Kg^{-1}, gills 389.4 mg Kg^{-1} and 1401.2 mg Kg^{-1} in cartilage [2]. Similar studies were performed in prawns, where maximum fluoride deposition was observed in skeletal tissues and least in soft tissues. Fluoride was accumulated within the duration of 4 days and was completely depurated within a period of 14 days [14]. Similar experimental results were observed in fresh water fish, Catla and a marine water fish, Sardine [15].

The degree and rate of depuration depends on different chemicals used, which also reflects the chemical concentration present in the media [16]. Toxicokinetic pattern of arsenic was studied in *Danio rerio*, where they exposed the fishes to arsenic for 60 days. Maximum bioaccumulation was observed in gills and then in liver and gonads. Depuration pattern results showed 100% depuration was obtained in whole body, liver

and gills of fishes in a period of 7 days. However, 10 days were utilized for depuration in ovaries [17]. In another study, accumulation and depuration of cobalt were observed in fresh water fish, *Capoeta fusca*, maximum accumulation was observed in liver tissue and depuration was attained maximum in gill tissues [18]. Another study was conducted by Mok et al. [19] to compare the accumulation of paralytic shellfish poison (PSP) in various marine creatures. When comparing the highest PSP concentrations in the different bivalve species, blue mussels had greater PSP levels than bay scallops, oysters, and short neck clams. The level of PSP in the digestive gland dropped by roughly 50% after one day and by roughly 77% after seven days in the blue mussel after depuration. In contrast, depuration had little effect on the PSP levels in the soft body, gill, or mantle.

In the present study maximum accumulation (after bone) was found in liver sample, wherein we observed maximum depuration rate of 100%. Similarly, Nile Tilapia (Oreochromis niloticus) was subjected to a chronic exposure of 28 days to observe effect of increasing water temperature and ambient concentration on fish's accumulation and removal of mercury. The experiment lasted for 28 days. The results showed that increased accumulation in the liver and kidney caused by higher water temperature and ambient mercury. Higher water temperatures caused a higher rate of mercury elimination. The Liver and kidney appeared to be the preeminent organs for accumulating mercury. Decontamination of Hg was found higher in liver and kidney tissues, however minimum depuration was observed in flesh [20].

CONCLUSION

Toxicokinetics analyses aid in understanding the mechanism of action of the chemical and/or its metabolites as well as the relationship between the chemical concentration/dose and the observed toxicity impact. Such toxicokinetics analyses are performed in a number of industries, including pharmaceuticals and food additives as well as pesticides and biocides, cosmetics, environmental contaminants, and industrial and occupational health sectors. Analyzing and examining toxicokinetics pattern of NaF on model organism, *Clarias batrachus* is a novel work in the field of toxicology.

A highly significant and direct relationship of fluoride accumulation has been observed with the concentration of NaF in the exposure medium. This provides an effective tool for monitoring environmental toxicity. Maximum fluoride accumulation has been estimated in bone tissue. Depuration was observed highest in liver samples of exposed fishes, which may be because liver is an organ of detoxification, which eliminates toxins out of the body.

Conflict of Interest. The authors declared that there is no conflict of interest.

Authorship Contributions. Concept: P.M., A.P., Design: P.M., Data Collection or Processing: P.M., Analysis or Interpretation: P.M., A.P., Literature Search: P.M., A.P., S.G., Writing: P.M., S.G.

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