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Combined Toxicity and Bioconcentration of Fluoride and Arsenic in African Catfish *Clarias gariepinus* (Burchell, 1822)

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Abstract— *Laboratory experiments were performed to examine the combined toxic effects of two important aquatic contaminants viz., arsenic and fluoride on African catfish, Clarias gariepinus. Additionally, the bio concentration factors (BCFs) of the two contaminants in tissues and blood of catfish were also determined. The LC_{50} for sodium fluoride and arsenic trioxide were determined to be 619.3 mg L^{-1}, 30.3 mg L^{-1}, respectively. Erratic swimming movements with hyperactivity, loss of equilibrium, augmented air gulping and decreased food consumption were observed in the experimental groups. In co-exposure groups of arsenic and fluoride, the concentration of fluoride in fish tissues increased with increasing water fluoride concentration in the test aquaria with significant differences ($P<0.01$) between different groups. Also significant differences ($P<0.05$) in tissue concentrations of arsenic between groups were observed in response to different concentrations of water arsenic. However, the differences in blood fluoride and arsenic concentrations were not significantly dissimilar ($P>0.05$) among the exposure groups. Arsenic was observed to exceedingly bioaccumulate and biomagnify in the tissues. Perhaps due to the complex formation of arsenic and fluoride the bio concentration of arsenic in tissues was observed to decrease with increasing water fluoride concentration and vice-versa. The study concludes that fluoride may interfere with the bio-concentration of arsenic.*

Keywords— Arsenic, Bio-concentration factor, Combined toxicity, Fluoride, LC_{50}.

I. INTRODUCTION

Fluoride and arsenic are two stern drinking water contaminants recognized worldwide [1] with natural sources contributing to the bulk of their environmental load. Fluoride is found in freshwater at concentrations less than 1.0 mg L^{-1}; however, its natural concentrations may exceed even 50.0 mg L^{-1} [2]. While lower concentrations, viz., <1.0 mg L^{-1} according to Bureau of Indian standards and 1.5mg L^{-1} according to World Health Organization are beneficial, higher concentrations may lead to various health problems [1]. Fluoride causes fluorosis, a slow degenerative disease affecting teeth and bone tissues. It also induces neurological defects, infertility, mental retardation, depression of thyroid activity [3, 4, 5, 6, 7] and persistently bioaccumulates in aquatic animals continuously exposed to the contaminated medium [8, 9, 10].In India, 19 out of 35 states and union territories have ground water highly contaminated with fluoride [11].

A heavy metal, arsenic is more toxic than fluoride at the same dose and exposure duration [1]. Symptoms of toxicity during short term exposures in humans include vomiting, abdominal pain, encephalopathy, and watery bloody diarrhea. Long-term exposure may result in thickened pigmented skin, abdominal pain, diarrhea, heart disease, numbness, and cancer. Globally, arsenic toxicity is mostly prevalent in West Bengal (India), Nepal, and Bangladesh [12, 13] with contaminated drinking water being the most common source. A higher concentration of arsenic is lethal to many organisms in the aquatic environment [14, 15] inducing the synthesis of stress related proteins [16] and alterations in B and T cell functions [17] in the fish body. Like other heavy metals, it is non degradable and considered hazardous to aquatic ecosystem due to its environmental persistence and tendency for bioaccumulation [18, 19, 20]. Donohue and Abernathy [21] reported that total arsenic (µg g^{-1} dry weight), in marine fish, shellfish, and freshwater fish tissues ranged between 0.19 to 65, 0.2 to 125.9, and 0.007 to 1.46, respectively.

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Koch et al., [22] demonstrated that total arsenic in freshwater fish ranged from 0.28 to 3.1 for whitefish (*Coregonus clupeaformis*), 0.98 to 1.24 for sucker (*Catostomus commersoni*), 0.46 to 0.85 for wall eye (*Stizostedion vitreum*), and 1.30 to 1.40 µg g^{-1} dry wt. for pike (*Esox lucius*).

Geological structures and expanding human activities contribute to the high concentrations of both fluoride and arsenic. Although, their concurrent chronic poisoning is a sprouting disease prevalent in India and many other countries, however, few reports exist suggestive of their chronic co exposure [23]. Li et al., [24] studied effects of arsenic-fluoride co-exposure on rat teeth and observed no effects on dental tissues. Distinct damage on the nervous system of the offspring with decreased learning and memory ability was reported by Zhang et al., [25]. Altered histology of cerebral hemisphere subsequent to combined arsenic-fluoride exposure was observed by Chinoy and Shah [26] with arsenic having more prominent effects as compared from fluoride. Marked genotoxic effects were apparent in case of combined exposure to arsenic and fluoride as compared to their individual exposures [27, 28]. Exposure of mice to higher doses of fluoride and arsenic revealed their antagonistic effects [29] while, low doses showed synergistic effects [30]. González-Horta et al. (2015) [31] studied urinary arsenic and fluoride in human residents of Chihuahua, Mexico exposed to concurrent arsenic and fluoride in drinking water. Positive correlations between As and F in drinking water and between urinary arsenic and fluoride were observed.

Fishes, the major source of protein in many countries [32] are often contaminated with high concentration of water borne pollutants and act as a major vector for contaminant transfer to humans. Their ability to detect sudden changes in the environment and to monitor short or long term changes in water quality, make them efficient biomarkers. Although, toxic effects of elevated levels of fluoride [33, 34, 35, 36] and arsenic [16, 17, 37, 38] individually on various aquatic species are well documented, however, no work has been done on their combined toxic effects. The present study includes the determination of the Median Lethal Concentrations, bio concentration and behavioral effects of fluoride and arsenic separately and in combination on the freshwater fish *Clarias gariepinus* (Burchell, 1822).

II. MATERIALS AND METHODS

2.1. Determination of Median lethal Concentration (LC_{50}) of Fluoride and Arsenic

2.1.1. Experimental design

Seventy African catfish (*Clarias gariepinus*) of either sex weighing between 100 and 250 g were procured live from local hatcheries in Raipur. The animals were housed in an air conditioned animal house at 24 ± 2°C under 12 hours of light and dark cycles and acclimatized for a period of seven days using de-chlorinated tap water. Feeding was done with Tokyo fish food.

2.1.2. LC_{50} estimation of Arsenic and Fluoride

Post acclimation, the fishes were divided into seven groups of ten fish each and acute toxicity bioassay conducted by exposing the fish to diverse concentrations (100, 200, 300, 600, 800, 1000, 1200ppm) of sodium fluoride in glass aquaria. Similar experiments were conducted with 7 different concentrations (10, 20, 25, 40, 45, 60 ppm) of arsenic trioxide. The control group was kept in an aquarium having tap water without addition of sodium fluoride and arsenic trioxide. The bioassay was conducted in a static system. Mortality was recorded at every 24, 48, 72 and 96 hour of exposure. LC_{50} was calculated by Probit analysis using SPSS 16.0 [39].

2.2. Combined arsenic and fluoride toxicity assay

On the basis of 96 hr LC_{50} values and the 95% confidence limits of sodium fluoride and arsenic trioxide obtained from the preliminary tests, various concentrations viz., Group I (600ppm F+10ppmAs), Group II (350ppm F+20ppm As), Group III (600ppm F+20ppm As) and Group IV (350ppm F+40ppm As) of sodium fluoride and arsenic trioxide were selected for combined toxicity testing. Blood and tissue (liver, kidney and muscle) samples were collected at the end of 96 hours along with water samples for quantitative analysis of fluoride and arsenic. Behavioral changes during the 96 hours duration were also recorded. Physicochemical properties of the test water during exposure were measured according to standard methods. Water quality during experiment varied as follows: Ambient temperature 24-26°C, Water temperature 21-22°C, pH 7.5-8.5, Conductivity 350-460 µs cm^{-1}, Ammonia nitrogen 0.005-0.01 mg L^{-1}, TDS 200-250 mg L^{-1}.

2.3. Arsenic analysis

Water was collected in polyethylene vials and kept in refrigerator at 4°C until further analysis. 100 ml aliquots of water samples were taken in 250 ml beaker, covered with watch glass and digested with concentrated HNO_3 on a hot plate [40].Blood was collected in EDTA coated tubes and stored in refrigerator at 4°C until further analysis. 0.5 ml blood was taken in 100 ml conical flask, covered with watch glass and digested by the addition of 5 ml conc.HNO_3: $HClO_4$ (6:1) on a hot plate [40]. Liver, kidney and muscles were isolated and dried in an oven at 100°C for

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24 hours. 0.5 g dried tissue samples were taken in 100 ml beaker. The sample was covered with watch glass and digested on a hot plate by the addition of HNO_3: $HCLO_4$ (3:1) [41].All the digested samples were filtered through Whatman filter paper No. 541 and diluted to 25 ml using distilled water. Sample analysis was done by Thermo Fisher Scientific Atomic Absorption Spectrophotometer (Model no.ICE 3000).

2.4. Fluoride analysis

Collection and storage of all samples was done as mentioned in arsenic analysis. Water fluoride was measured by direct determination method by adding TISAB buffer (1:1) .Tissue samples were digested with a mixture of 1:1 (HNO_3: $HCLO_4$) and neutralized with citrate buffer. Final sample solution was obtained by adding TISAB buffer (1:1). Fluoride content was measured by direct determination method [42] with required modifications. Blood fluoride was measured by Analyte addition method using the following equation (Thermo scientific Orion ion selective electrode manual):

$$\mathbf{C_U = C_S\,[(V_U / V_{S+}V_U)* 10^{\Delta E/S}]}$$

Where: $\mathbf{C_U}$ = concentration of unknown sample, $\mathbf{C_S}$ = concentration of standard sample, $\mathbf{V_U}$ = volume of unknown sample, $\mathbf{V_S}$ = 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

In all samples, quantification of F^- ion was done with the help of Thermo Fisher Scientific Orion 9609 BNWP ion selective fluoride electrode.

2.5. Bioconcentration factor (BCF): Bio-concentration factors of arsenic and fluoride in the fish samples were obtained using following equation:

$$BCF = C_{org} / C_{water}$$

Where BCF- bio-concentration factor, C_{org} = concentration of chemical in aquatic organism, C_{water}= concentration of chemical in ambient environment, water in this case.

III. RESULTS

No fishes were observed dead in the control aquarium at the end of the experiments. Highest fluoride concentration caused mortality with increasing exposure time. 100% mortality was observed at 1200 ppm, 60 ppm and 40+350 ppm for fluoride, arsenic and arsenic+ fluoride, respectively.

3.1. Determination of LC_{50} value of sodium fluoride for *Clarias gariepinus*

The observed percentages of mortality of *Clarias gariepinus* for sodium fluoride are shown in Tables 1, 2, 3 &4. The observed LC values and 95% confidence limits for LC_{25} (333.445-590.361), LC_{45} (454.322-743.495), LC_{75} (649.953-1141.940), LC_{96} (938.671-2279.511) are shown in Table 4. In this study, 96 hour LC_{50} of sodium fluoride on *Clarias gariepinus* was estimated to be 619.3 mg L^{-1}.

3.2. Determination of LC_{50} value of arsenic trioxide for *Clarias gariepinus*

The observed percentages of mortality of *Clarias gariepinus* for arsenic trioxide are shown in Tables 5, 6, 7 & 8. The observed LC values and 95% confidence limits for LC_{25} (13.5-29.2), LC_{45} (20.8-35.9), LC_{75} (31.8-59.39.0), LC_{96} (44.3-148.4) are shown in Table 8. In the present study, the 96 hour LC_{50} value of arsenic trioxide on *Clarias gariepinus* was estimated to be 30.3 mg L^{-1}.

3.3. Concentration of fluoride and arsenic in fish sample

The levels of arsenic and fluoride obtained in fish tissues and blood are depicted in Figures 3 and 4, respectively. Bio-concentration factors of arsenic and fluoride in blood and tissues are depicted in Fig. 5. Fluoride concentration in fish tissues increased with increasing water fluoride concentration in the test aquaria and significant differences exist ($P<0.01$) between different groups. Similar to fluoride, arsenic concentration increased in tissues with increasing water fluoride and arsenic concentration and accumulation in liver was higher than blood. Significant differences ($P<0.05$) in tissue concentrations of arsenic was observed in response to different concentrations of water arsenic levels. However, the differences in blood fluoride and arsenic concentrations were not significantly different ($P>0.05$) among the exposure groups. Bioaccumulation of arsenic in tissues was observed decreased by increasing water fluoride concentration. Similarly, bioaccumulation of fluoride in tissue was observed to decrease with increasing water arsenic concentration.

IV. DISCUSSION

Highly variable 96 hour Median Lethal Concentration (mg L^{-1}) values have been reported for fluoride in diverse species of fishes including *Oncorhynchus mykiss* (107.5) and *Salmo trutta* (160.5) at a water temperature between 15-16ºC [43, 44]; *Labeo rohita* (935) [45] and *Oreochromis mossambicus* (54.0)[46] at a water temperature of 18–30ºC and *Puntius sophore* (126.12) [48] at 19-20 ºC . The 96 hour LC_{50} of sodium fluoride on *Clarias gariepinus* (619.3 mg L^{-1} at 21-22 ºC of water temperature) obtained in this study undoubtedly, proves the hardy nature of *Clarias gariepinus* which appears to be far more tolerant to fluoride

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as compared from *Oreochromis mossambicus, Oncorhynchus mykiss, Puntius sophore and Salmo trutta.*

On the basis of 96 hours LC_{50} values (30.33 mg L^{-1}) observed in this study for arsenic, *Clarias gariepinus* appears to be equally tolerant than *Oryzias latipes* (30) [47] and *Cyprinus carpio* (32) [49], less tolerant than *Ctenopharyngodon idella* (89) [50] and *Channa punctatus* (76) [16] and more tolerant than *Carassius carrassius auratus* (10), *Anabas testudineus* (18.21) [51], *Danio rerio* (8.91) [52] and *Clarias batrachus* (8.4) [17].Erratic swimming activity, increased opercular movement and mucous secretion, loss of equilibrium and body dispigmentation ,changes in feeding behavior, similar to reports by Bhavani and Karuppasamy [52], Narwaria and Saksena [48] were also observed.

Although numerous studies exist on evaluation of the individual effect of fluoride and arsenic on mammals and fishes, there are no studies related to understanding the potential combined effects of fluoride and arsenic on fish. Cao et al., [53] reported that the concentration of fluoride in the gills and other tissues in *C. carpio* increased with exposure time and exposure concentration and were in the order of gills > liver > brain > kidney > muscle > intestine. In this study, tissue fluoride content of *Clarias gariepinus* increased significantly ($P<0.01$) with increasing water fluoride and arsenic concentration and exposure time. Similar results were obtained by Aguirre-Sierra *et al.,* [54] after exposing white clawed crayfish (*A. pallipes*) to different concentration of fluoride. Our results also depict BCF values of blood arsenic (0.48 - 0.50), blood fluoride (0.024 - 0.384) and tissue fluoride (0.58 - 0.87), to be in the lower range ie. (<01). However, tissue arsenic was observed to be very high (0.63 - 3.11), indicating that arsenic exceedingly bioaccumulated and biomagnified in the tissues. Data show bioaccumulation of arsenic and fluoride to occur predominantly in liver, it being responsible for detoxification and elimination of toxic elements. Differences in blood fluoride and arsenic concentrations were not significantly different ($P>0.05$) among the exposure groups.

Bio concentration of arsenic in tissues was observed to decrease with increasing water fluoride concentration and vice-versa. We agree with [23] that the possible reason of this antagonistic behavior could be the presence of an empty *d* orbital of fairly low energy in arsenic which predominately binds with the halogen due to its electro negativity. In trivalent oxidation state it shows SP_3 hybridization with the formation of AsF_3, while in pentavalent oxidation state it shows SP_3d hybridization and forms AsF_5 which is a potent ion acceptor forming AsF_6^- ions or more complex species. Hence, fluoride possibly suppresses the ionization of sodium arsenite thereby reducing its retention.

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VI. DECLARATION OF INTEREST

The authors declare that there are no conflicts of interest to disclose.

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TABLES

Table.1. *Parameter Estimates for fluoride vs. Clarias gariepinus*

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Probit[a]	Concentration	5.769	1.278	4.513	.000	3.264	8.275
	Intercept	-16.107	3.584	-4.494	.000	-19.691	-12.523

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Table.2: Log concentration, observed responses in fish Clarias gariepinus

Number	Concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
1	2.000	10	0	.000	.000	.000
2	2.301	10	0	.023	-.023	.002
3	2.477	10	0	.347	-.347	.035
4	2.602	10	0	1.367	-1.367	.137
5	2.699	10	5	2.960	2.040	.296
6	2.778	10	6	4.684	1.316	.468
7	2.903	10	7	7.394	-.394	.739
8	3.000	10	8	8.851	-.851	.885
9	4.079	10	10	10.000	.000	1.000

Table.3: Confidence Limits for fish Clarias gariepinus

Probibility Probit point	95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
	Concentration	Lower Bound	Upper Bound	Concentration	Lower Bound	Upper Bound
LC1	244.701	112.764	343.699	2.389	2.052	2.536
LC25	473.113	333.445	590.361	2.675	2.523	2.771
LC45	588.976	454.322	743.495	2.770	2.657	2.871
LC50	619.269	484.202	789.417	2.792	2.685	2.897
LC75	810.576	649.953	1141.940	2.909	2.813	3.058
LC96	1245.487	938.671	2279.511	3.095	2.973	3.358
LC99	1567.196	1119.693	3366.831	3.195	3.049	3.527

a. Logarithm base = 10.

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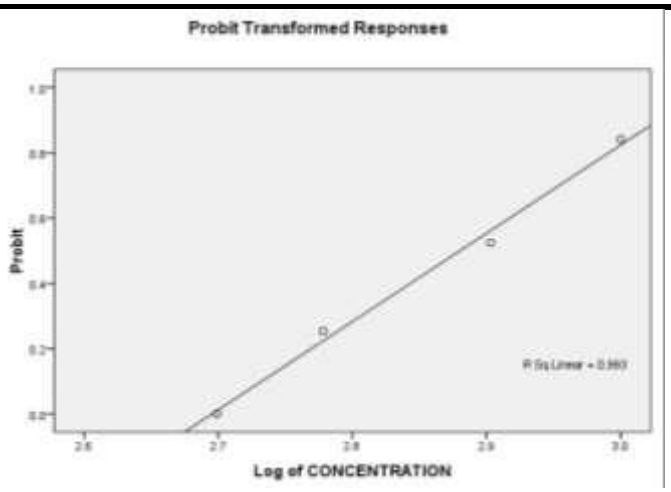

Fig. 1: The graph showing linear curve between Log concentrations of sodium fluoride against probit mortality of fish Clarias gariepinus

Table.4: Parameter Estimates for arsenic vs. Clarias gariepinus

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Probit[a]	Concentration	6.110	1.845	3.312	.001	2.495	9.726
	Intercept	-9.056	2.795	-3.240	.001	-11.851	-6.260

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Table.5: Log concentration, observed responses in fish Clarias gariepinus

Number	Concentration	Number Of Subjects	Observed Responses	Expected Responses	Residual	Probability
1	1.301	6	1	.807	.193	.134
2	1.398	6	2	1.823	.177	.304
3	1.602	6	3	4.611	-1.611	.768
4	1.653	6	6	5.114	.886	.852
5	1.778	6	6	5.789	.211	.965

Table.6: Confidence Limits for fish Clarias gariepinus

Probibility Probit Point		95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
		Concentration	Lower Bound	Upper Bound	Concentration	Lower Bound	Upper Bound
	LC1	12.626	3.133	18.568	1.101	.496	1.269
	LC25	23.528	13.540	29.175	1.372	1.132	1.465
	LC45	28.934	20.762	35.956	1.461	1.317	1.556
	LC50	30.337	22.603	38.210	1.482	1.354	1.582
	LC75	39.116	31.793	59.390	1.592	1.502	1.774
	LC96	58.678	44.318	148.423	1.768	1.647	2.172
	LC99	72.893	51.518	248.919	1.863	1.712	2.396

Table.7: Behavioral changes in Clarias gariepinus after exposure to various concentration of fluoride and arsenic

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Parameter	**Control group**	**F^- exposed group**	**As exposed group**
Body position	Horizontal at the bottom of the aquarium	Both horizontal and vertical	At the bottom of the aquarium
Operculum movement	2 per 5 minutes	6-7 per 5 minutes	7-8 per 5 minutes
Sensitivity to food	Immediate	Slow	Initially unresponsive then sluggish
Swimming movements	Normal	Erratic	Erratic

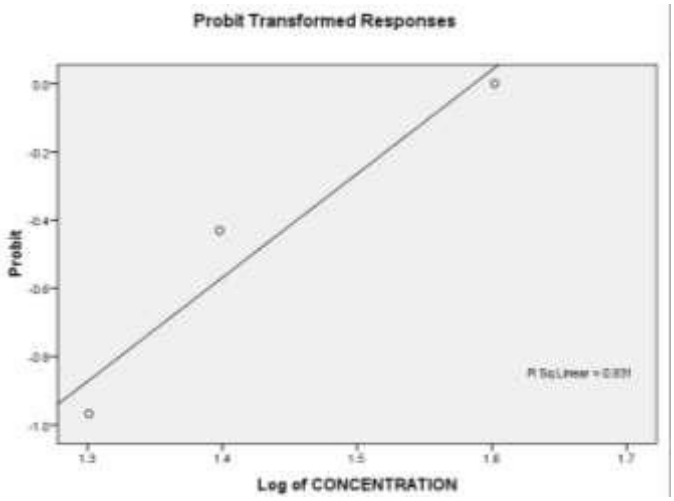

Fig. 2: *The graph showing linear curve between Log concentrations of arsenic trioxide against probit mortality of fish Clarias gariepinus*

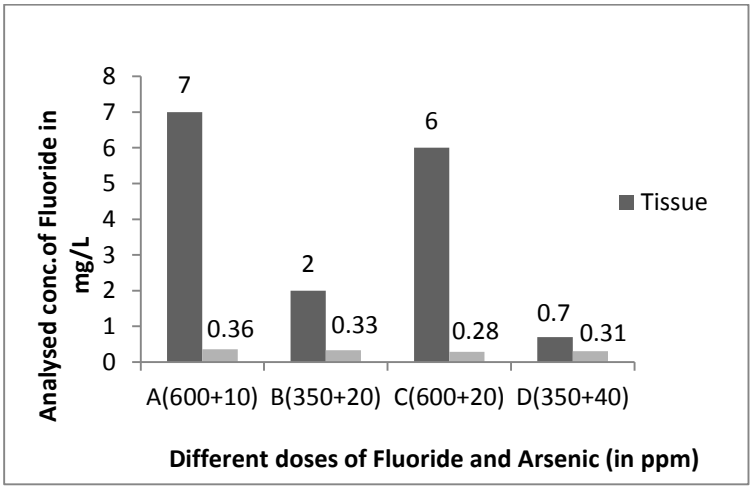

Fig. 3: Comparison in the concentrations of arsenic in blood and tissue of Clarias gariepinus.

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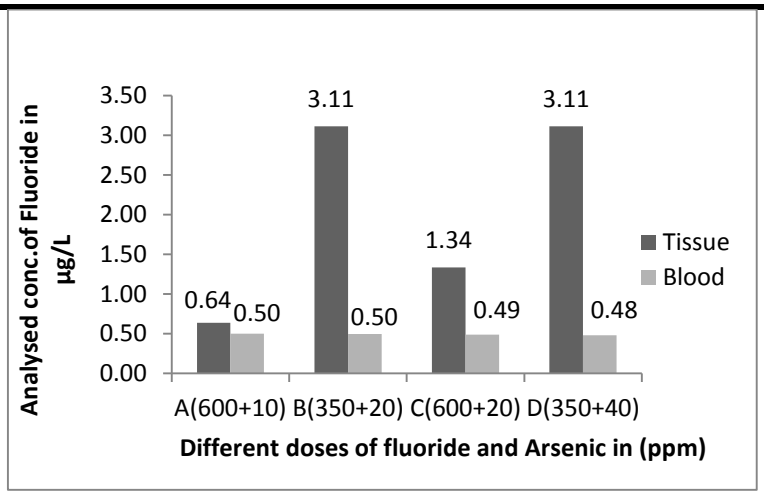

Fig. 4: Comparison in the concentrations of fluoride in blood and tissue of Clarias gariepinus

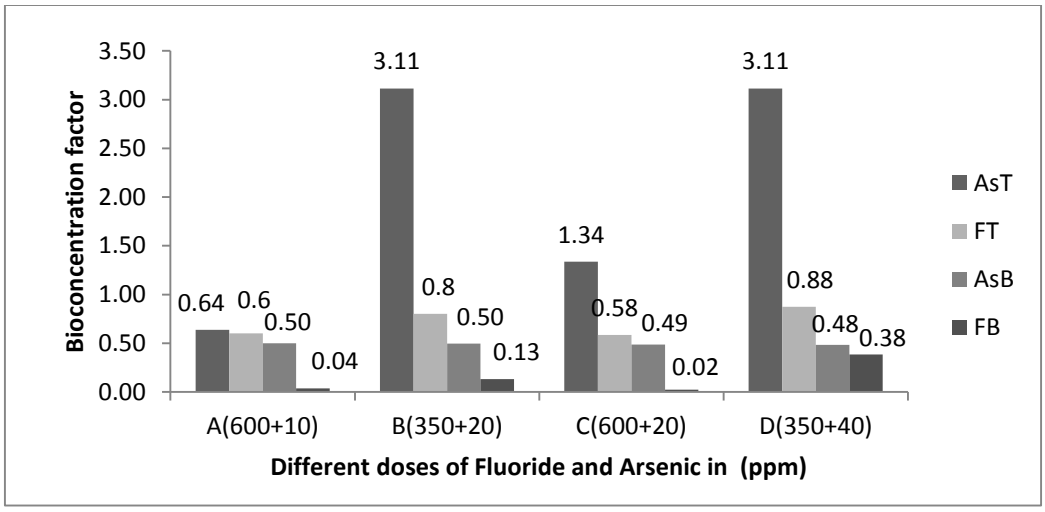

Fig.5: Comparison in the bio concentration factors of arsenic and fluoride in blood and tissues of Clarias gariepinus