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## Estimation of fluoride bioaccumulation in different tissues of *Channa punctatus* from water bodies of Lucknow region

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### Abstract

Fluoride is ubiquitously present in the environment. Thus problem related to fluoride toxicity has become a major issue all over the world, as its level in water, soil and air is gradually getting higher due to urbanization and modernization. Fluoride level in drinking water has now increased more than the maximum desirable level prescribed by W.H.O. studies. Fluoride is one of a wide spread crippling scourge all over the world. Its ingestion in drinking water causes skeletal as well as dental fluorosis. In India, the most seriously affected states are Andhra Pradesh, Punjab, Haryana, Rajasthan, Uttar Pradesh, Gujarat, Tamil Nadu, Bihar and Maharashtra. Excessive concentration of fluoride is not only seriously affecting the human health but it also causes a serious problem to the aquatic ecosystem. Fresh water ecosystem is affected due to low concentration of magnesium and calcium ion, which combine with fluoride ions to form their salts of fluorides, which is less toxic in comparison to free fluoride ions. Excessive ingestion of fluoride accumulates and damages organs like liver, kidney, gills and other vital organs.

Present study is to investigate the bioaccumulation of fluoride in different tissues of fresh water fish *Channa punctatus* after exposure to fluoride at various concentration levels. It was noticed that fluoride was taken rapidly by bony tissues which are skeletal, operculum and scales. However, in soft tissues such as liver, kidney, stomach, intestine, gill and muscles, bioaccumulation of fluoride were found to be accumulated but to less extent.

**Keywords:** *Channa punctatus*, bioaccumulation, fluoride toxicity

### Introduction

Fluoride is one of the most useful elements for the development of skeleton system. On the other hand, excessive fluoride exposure leads to various structural and functional impairment both in animals and human beings. In recent years occurrence of excess fluoride in aquatic system is of much concern because of its toxicity and several adverse physiological consequences in the fluoride endemic areas. The problem of fluoride toxicity is much severe in freshwater ecosystem because of low concentration of calcium and magnesium ion which combine with fluoride ion to form their salts of fluoride ions. The highest natural fluoride concentration (2800mg per litre) ever found in water was recorded in lake Nakuru in the Rift valley in Kenya, (Murray 1986).

In India majority of the human population depend upon various aquatic organisms for their nutritive purpose such as crabs, prawn, molluscs and fishes etc. FAO data of 2001 reveals that fisheries products are major source of food for the population all over the world. It is the major of protein and it also provides vitamin A, vitamin D and large amount of phosphorus and other elements. They are cheaper source to overcome malnutrition which is a major problem of developing countries.

Since last few decades extensive use of chemicals in industries on one hand have opened new dimension of development in country but on the other hand destroyed our ecosystem, particularly water bodies. Water bodies are natural habitat of aquatic animals and are now being polluted by these chemicals. Several studies have reported that insecticides, pesticides, detergent and fertilizer affect the physiology of the aquatic organisms Kumar *et al.* (1998, 1999) have reported deltamethrin, synthetic pyrethroid induced physiology changes in *Heteropneustes fossilis*. Das *et al.* (1994) [7] exhibited the effect of pesticides on some biochemical constituents of fresh water teleost *Channa punctatus*. Similarly Asztalos *et al.*

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(1990) [2], Dutta *et al.* (1994) [8], Khare and Singh (2002) [10] have reported alteration in biochemical constituents in various organs after exposure to certain pesticides, malathion and certain toxic chemicals such as Hg, Cd, Zn and Pb etc in fishes. In present study an attempt has been made to evaluate the toxic effect of fluoride on soft and bony tissues of fresh water fish *Channa punctatus* which is available abundantly in local areas.

### Materials and Methods

Healthy fresh water fish *Channa punctates* were collected from the local resources and were brought to the laboratory. Fishes were given a treatment in 1% potassium permagnate and acclimatized in the glass aquaria for period of two weeks. During acclimatization they were maintained in chlorine free fresh water with continuous aeration. The physico chemical properties of the water were determined by standard method (APHA, 1998) [1]. PH  $6.9 \pm 0.02$ , temperature (C)  $26 \pm 2$ , dissolved oxygen  $8.8 \pm 2.5$ , alkalinity (mg/l) 90-100, water hardness (mg/l) as  $\text{CaCO}_3$  118-120. Water was changed every alternate day and fishes were fed once a day ad libitum with dry fish and goat liver pieces. For experiment, fishes were divided into three groups, each group contains 10 fishes. Group I served as control, while Group II and III served as experimental group and were exposed to fluoride. Group II was treated with low dose (30mg/l) and group III was treated with high dose (60mg/l) of fluoride. The source of the fluoride was sodium fluoride (NAF; Excel R, India limited Mumbai). Experiment was conducted for three months (90 days) with renewal of water at the alternate days.

After completion of exposure period, both exposed and control fish were dissected. Sample of the soft s bony tissues were collected and analyzed for the bioaccumulation in different soft and bony tissue such as liver, kidney, gill, intestine, oesophagus, stomach, muscle, skin, operculum, scales and skeleton were assessed by the method of Birkeland (1970) [3]. Skeleton, operculum scales were ashed at  $500^\circ\text{C}$  in furnace and other soft tissues were homogenised as such. After homogenization and drying of 24hrs at  $105^\circ\text{C}$  about 100mg of the dry sample was dissolved in acid (equal parts of 11.6M perchloric acid and 14.3M Nitric acid) and then neutralized and buffered to PH  $5.2 \pm 5.5$  with an alkaline mixture of 7.8 sodium hydroxide (NaOH) and 1.0m tri-sodium chlorate. Dissolution and buffering took place in close chamber. After that fluoride level was determined by specific ion analyzer (Orion EA940) using fluoride electrode. At least 6 reading of each sample were taken out.

### Result and Discussion

Bioaccumulation of fluoride was observed in different soft and bony tissues of *C. punctatus* such as liver, kidney, stomach, intestine, muscles, gills, operculum, scale, skin and skeleton after exposure to sub lethal concentrations of fluoride i.e 30mg/l and 60mg/l. The level of fluoride was significantly elevated in all the tissues.

**Liver:** In liver of control group of fish fluoride level was found to be  $2.61 \pm 0.11 \mu\text{gF}/100\text{mg}$  while at lower concentration of fluoride level was noticed to be  $3.06 \pm 0.02 \mu\text{gF}/100\text{mg}$  and at higher concentration of fluoride level was observed to be  $7.17 \pm 0.06 \mu\text{g F}/100\text{mg}$  as compared to control group. The fluoride level was increased by 17.2% at lower concentration and 174.7% at higher concentration of fluoride as compared to control group. (Table-1; Fig-1)

**Kidney:** In kidney of control group of fish fluoride level was noticed to be  $8.33 \pm 0.15 \mu\text{gF}/100\text{mg}$ . However at lower concentration of fluoride level was observed to be  $16.11 \pm 0.10 \mu\text{gF}/100\text{mg}$  and at higher concentration of fluoride level was found to be  $18.73 \pm 0.13 \mu\text{gF}/100\text{mg}$  as compared to control group. The increasing percentages were 93.3% and 124.8% in lower and higher concentration of fluoride as compared to control group. (Table-1; Fig-1)

**Stomach:** In stomach fluoride level in control group was observed to be  $5.29 \pm 0.18 \mu\text{gF}/100\text{mg}$  and after exposure of fish to lower concentration 30mg/l it was found to be  $6.34 \pm 0.16 \mu\text{gF}/100\text{mg}$  and at higher concentration 60 mg/l) it was noticed to be  $9.30 \pm 0.18 \mu\text{gF}/100\text{mg}$  as compared to control group the fluoride level was increased by 19.8% at lower concentration and 75.8% at higher concentration of fluoride as compared to control group. (Table-1; Fig-1)

**Intestine:** In intestine fluoride level in control group was found to be  $7.54 \pm 0.12 \mu\text{gF}/100\text{mg}$ . However at lower concentration it was noticed to be  $72.53 \pm 0.59 \mu\text{gF}/100\text{mg}$  and at higher concentration of fluoride level was observed to be  $15.31 \pm 1.26 \mu\text{gF}/100\text{mg}$  as compared to control group. (Table-1; Fig-1)

**Muscles:** In muscles fluoride level in control group was found to be  $22.88 \pm 0.57 \mu\text{gF}/100\text{mg}$ . However at lower concentration it was noticed to be  $13.4 \pm 0.17 \mu\text{gF}/100\text{mg}$  and at higher concentration of fluoride level was observed to be  $62.03 \pm 0.75 \mu\text{gF}/100\text{mg}$  as compared to control group. (Table-1; Fig-1)

**Gills:** In gills fluoride level in control group was observed to be  $37.58 \pm 0.49 \mu\text{gF}/100\text{mg}$ . However at lower concentration it was noticed to be  $66.82 \pm 1.00 \mu\text{gF}/100\text{mg}$  and at higher concentration of fluoride level was observed to be  $92.71 \pm 2.20 \mu\text{gF}/100\text{mg}$  as compared to control group. (Table-1; Fig-1)

**Operculum:** In operculum fluoride level in control group was found to be  $55.54 \pm 0.95 \mu\text{gF}/100\text{mg}$ . However at lower concentration it was noticed to be  $104.1 \pm 1.51 \mu\text{gF}/100\text{mg}$  and at higher concentration of fluoride level was observed to be  $126.02 \pm 1.63 \mu\text{gF}/100\text{mg}$  as compared to control group. (Table-1; Fig-1)

**Scales:** In scales fluoride level in control group was observed to be  $74.36 \pm 0.27 \mu\text{g F}/100\text{mg}$ . However at lower concentration it was noticed to be  $149.18 \pm 1.90 \mu\text{gF}/100\text{mg}$  and at higher concentration of fluoride level was observed to be  $241.0 \pm 0.66 \mu\text{gF}/100\text{mg}$  as compared to control group. (Table-1; Fig-1)

**Skin:** In skin fluoride level in control group was found to be  $20.7 \pm 0.11 \mu\text{gF}/100\text{mg}$ . However at lower concentration it was noticed to be  $52.14 \pm 0.33 \mu\text{gF}/100\text{mg}$  and at higher concentration of fluoride level was observed to be  $82.62 \pm 1.52 \mu\text{gF}/100\text{mg}$  as compared to control group. (Table-1; Fig-1)

**Skeleton:** In skeleton fluoride level in control group was found to be  $88.49 \pm 0.59 \mu\text{gF}/100\text{mg}$ . However at lower concentration it was noticed to be  $123.2 \pm 0.16 \mu\text{gF}/100\text{mg}$

and at higher concentration of fluoride level was observed to be  $177.15 \pm 0.69 \mu\text{gF}/100\text{mg}$  as compared to control group. (Table-1; Fig-1)

So highest accumulation of fluoride was noticed in skin (299.1%) followed by scales (244.0%), muscles (177.1%) and liver (174.7%). But moderate amount were observed in gills (146.7%), operculum (126.8%) and kidney (124.8%) however lesser percentages of fluoride accumulation were found in intestine (103%) followed by skeleton (100.1%) and stomach (75.8%). Similar studies were reported by many workers in vertebrates and invertebrates (Neuhold and sigler 1960; Zipkin *et al.*, Ke *et al.* 1970; Moore 1971; Wright and Davison 1957; Hemens *et al.*, 1975, Pankhurst *et al.* 1980; Connel and Airey 1982; Oehlenschlaenger and Manthey 1982; McClurg 1984; Vogel and Ottow, 1991 and Camargo 2002) [14, 20, 12, 19, 16, 6, 11, 17, 5]. Neuhold and Sigler (1960) [14] observed the effect of NaF in Rainbow trout and *Cyprinus carpio*. They reported that skeleton bone accumulated much higher level of fluoride (range from 450-1100 mgF/kg wet wt). Oehlenschlaenger and Manthey (1982) [15] observed inorganic fluoride in fish *Oreochromis leucostictus* in muscles skin, gills and bone. They exhibited that bone accumulated highest percentage of fluoride. Kierdorf *et al.* (1989) have found mandibular fluoride in roe deer and positively correlated with age, the deer. Similarly Moore (1971) [12] noticed the uptake and content of fluoride was mainly accumulated in

exoskeleton ( $298 \mu\text{gF/g}$  dry wt) and less in muscles ( $10 \mu\text{gF/g}$ ). Wright and Davison (1975) [19] observed accumulation of fluoride by several marine crustaceans inhabiting the area close to an aluminium smelter. They found highest accumulation in exoskeleton. They further reported the tissue accumulation of fluoride in *Gadus morhua* and observed highest percentage of fluoride in skin ( $29.6 \text{ mgF/kg}$  wet wt) some aquatic invertebrates can accumulate relatively higher concentration of fluoride in soft tissues noticed by many investigators (Barbaro *et al.* 1981, Walton 1986 and Breimer *et al.* 1989) [18, 4].

Among the tissues which were in direct contact with the dissolved fluoride gill was found to accumulate more than intestine and stomach. This is because it is the primary route of uptake to fluoride. Uptake tissue distribution and persistence of fluoride in soft tissue, gill, muscle, liver, kidney, intestine and stomach may be due the role of blood circulation in transport of fluoride. Soluble fluorides are almost completely absorbed from the gastrointestinal tract; however the extent of absorption may be reduced by complex formation with aluminium, phosphorus, magnesium or calcium. In the hard tissue i.e. scales, operculum and skeleton the fluoride becomes incorporated into the crystal lattice form. From the present investigation it is evident that accumulation of fluoride in tissues of *C. punctatus* directly or indirectly alters the physiology of fish under toxic stress.

**Table 1:** Bioaccumulation of fluoride ( $\mu\text{gF}/100\text{mg}$ ) in different tissues of *C. punctatus* after exposure to sub lethal concentrations of fluoride (30mg/l) and (60mg/l) for the period of 90 days.

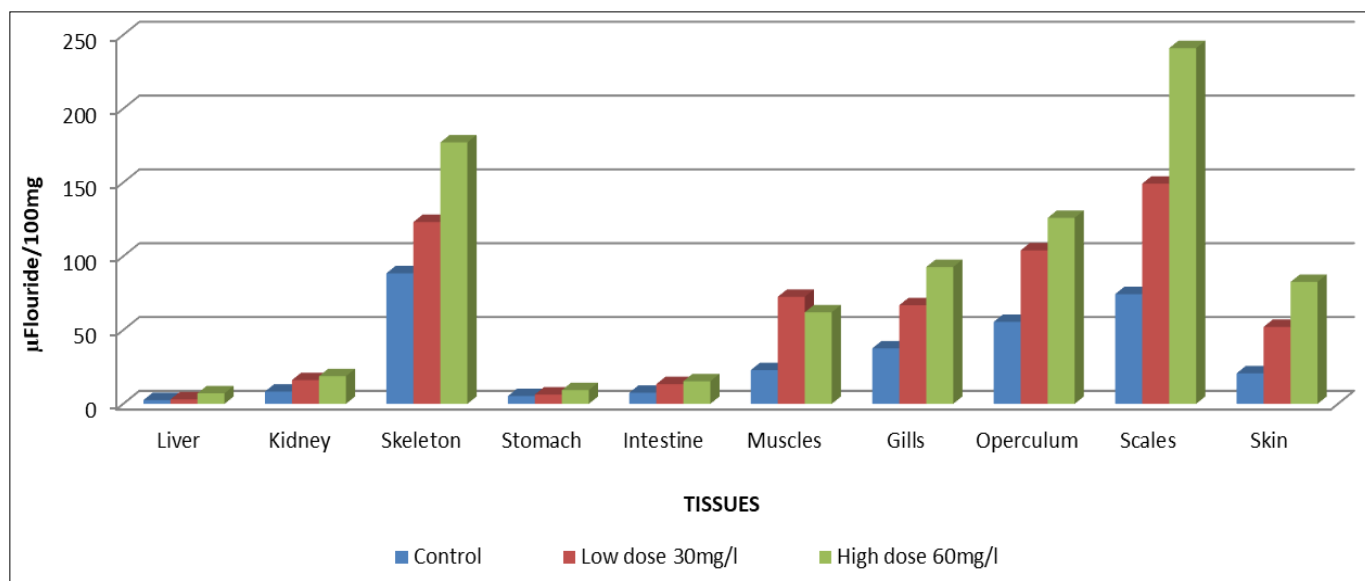
Tissues $\mu\text{gF}/100\text{mg}$	control	Low dose 30mg/l	% decrease	High dose 60mg/l	% Decrease
Liver	$2.61 \pm 0.11$	$3.06 \pm 0.02$	17.2	$7.17 \pm 0.06^*$	174.7
Kidney	$8.33 \pm 0.15$	$16.11 \pm 0.10^*$	93.3	$18.73 \pm 0.13^*$	124.8
Skeleton	$88.49 \pm 0.59$	$123.2 \pm 0.16^*$	39.2	$177.15 \pm 0.69^*$	100.1
Stomach	$5.29 \pm 0.18$	$6.34 \pm 0.16$	19.8	$9.30 \pm 0.18^*$	75.8
Intestine	$7.54 \pm 0.12$	$13.4 \pm 0.17$	77.7	$15.31 \pm 1.26^{**}$	103.0
Muscles	$22.88 \pm 0.57$	$72.53 \pm 0.59^*$	217.0	$62.03 \pm 0.75^*$	177.1
Gills	$37.58 \pm 0.49$	$66.82 \pm 1.00^*$	77.8	$92.71 \pm 2.20^*$	146.7
Operculum	$55.54 \pm 0.95$	$104 \pm 1.51^*$	87.4	$126.02 \pm 1.62^*$	126.8
Scales	$74.36 \pm 0.27$	$149.18 \pm 1.90^*$	100.6	$241.00 \pm 0.66^*$	224.0
Skin	$20.7 \pm 0.11$	$52.14 \pm 0.33^*$	151.1	$82.62 \pm 1.52^*$	299.1

Values are mean  $\pm$  S.E.

\* $P < 0.001$  (as compared to control group)

\*\* $P < 0.01$  (as compared to control group)

No. of observation in 6 fish



**Fig 1:** Bioaccumulation of fluoride in different tissues of *C. punctatus*

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