

STUDY ON TOXICOLOGICAL EFFECTS OF FLUORIDE ON FISH LABEO ROHITA MAHARAJA POND MAUGANJ REWA (M.P.)

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ABSTRACT: - Fish take up fluoride directly from water and are susceptible to fluoride contamination of their environment. Aquatic organisms living in soft water may be more adversely affected by fluoride pollution than those living in hard or sea water. In this study, we examined the tissue distributions of fluoride and its bioaccumulation in the gills, scales, muscles and bone of the fish *Labeo rohita* chronically exposed to fluoride. *Labeo rohita* was exposed to sub-lethal concentrations of fluoride (33.488 mg/l, 66.976 mg/l) for a period of 8 days and 15 days. The tissue of fish i.e., gills; scales, muscle and bone were collected. The fluorides accumulated in these tissues were analyzed by the Orion model 94-09 fluoride ion electrode consisting of an electrode made from crystal lanthanum. The fingerlings exposed to fluoride accumulated considerable amount of fluoride in different tissues. The accumulation is comparatively high in fish exposed to long duration. Fluoride accumulation is more in gills and scales and less in bone and muscle.

KEYWORDS -: Fluoride, *Labeo rohita*, Toxicity.

INTRODUCTION:-

Most rivers, streams, and ponds in India are severely polluted or serve as “open sewers” because domestic sewage and industrial wastes, either untreated or partially treated are discharged into them. They contain many toxic chemicals that adversely affect aquatic organisms. Owing to expanded industrial emissions and commercial uses of F compounds, the concentration of F is increasing in both ground water and surface water.

Fluoride toxicities in animals has become an important toxicological problem in many parts of the world due to the expansion of certain types of industrial operations that emit excessive fluorides into the atmosphere in areas

of animal habitat on land and in water. Animals normally ingest low levels of fluoride with no adverse effects. Small amount of fluoride may even have beneficial effects; but when excessive amount are is ingested for prolonged period of time, adverse effects are induced.

Acute fluoride toxicities have been studied and it is found variable to species. The responses seen in acute fluoride toxicities are varied and depend on many factors. Clinically the following signs may be observed; restlessness, stiffness, excessive mucus secretion and fast breathing rate.

One of the serious health problems faced by several countries is fluorosis affecting both human beings and livestock. Most rivers, streams and ponds in India are severely polluted; serve as open sewers because domestic sewage and industrial waste are discharged into them. Fluoride occurs in soils, water bodies and animal tissues. Animals ingest variable amount of fluoride throughout their lives. Its poisoning may result due to prolonged ingestion of fluoride above the threshold level. It is ingested maximum through water and food.

The toxic effect of elevated fluoride on various aquatic species is well documented (Gikunju 1992; Dwivedi et al. 1997; & Camargo 2003) of the fluoride absorbed half of it is retained in hard tissues and the remainder is rapidly excreted from the body (Largent 1961). Incorporation of the bone depends on the metabolic activity both in respect of age as well as the site in particular (Gardner et.al. 1950). Fluoride can replace carbonate or bicarbonate groups within or at the surface of crystallites by hetero ionic exchange (Newmann et.al. 1950) or it may be included in the apatite lattice during crystal formation “update by accretion. Chronic toxic effect results from the cumulative action of ingestion of smaller quantities of fluoride for long periods. The term

fluorosis is used to describe this toxic effect is by Moller and Gudjonason (1930).

There are number of reports from different parts of the country (Krishnamachary et. al. 1973). About 95% of total fluoride retained in the animal body is found in the bone, enamel, cement and dentine. This retention pattern has made it possible the establishment of certain criteria that are useful in evaluating the degree of fluorosis. The symptoms may vary considerably depending upon the amounts of fluoride ingested and the duration of intake.

Fluoride has a wide range of biological effects. In small quantities (i.e., 1 ppm) the inorganic fluorine prevents the decay of teeth both in human beings and animals. At level higher than 1 ppm of fluoride teeth and bone structure are adversely affected. Risk assessment for fresh water ecosystems and consequently for human health, however can be based partially upon surveying the pollutant concentration in water as it gives no information about the entrance of the substance into the food chain. Therefore, it seemed appropriate to extend the survey by examining the organisms and their ability to accumulate the fluoride pollutant in tissues. The carps grown in aquaculture ponds and streams have much significance since they are used as valuable food by human beings for large scale. These fish are capable of accumulating fluorides from natural waters having fluoride more than optimum levels (XiaotaoShi, RuifangWang, 2009). Hence an attempt has been made to study the accumulation of fluoride in selected tissues of *Label rohita*.

MATERIALS AND METHODS:-

Healthy living fish of size 8 to 10cm (fingerlings) and 500 to 800 mg were brought from local fish farm and acclimatized to laboratory conditions for 10 days. Such acclimatized fish were divided into two groups one group served as control and the other as experimental group. Such acclimatized fish were exposed to sub-lethal concentrations of fluoride i.e., 33.488 mg/l and 66.976 mg/l over a period of 8 days and 15 days as per APHA, 1998 Guidelines . After the said periods of exposure, the fish were randomly selected for fluoride analysis.

The Orion model 94-09 fluoride ion electrode consisting of an electrode made from crystal lanthanum was used. It was provided with pH meter and also an expanded scale

for measurement of the fluoride ion activity of the solutions. The standard graph was plotted by the method followed by Singer and Armstrong (1968) by making use of Sodium fluoride supplied by Merck Chemical Company, Bombay.

The fish were sacrificed on different tissues such as gills; muscle, scales and bone were collected and placed in an oven at 80⁰C until constant weight has been reached. They were ached at 550⁰C in a muffle furnace and the ash was weighed and fluoride was analyzed by the following method cited by Singer and Armstrong (1968).

Procedure: 4 to 6mg of bone ash was weighed in a smooth paper and transferred to a 50ml of polyethylene beaker and dissolved by addition of 1ml of 0.25N hydrochloric acid. The excess was neutralized by the addition of 1:1 ml of 0.12M Sodium hydroxide solution and a final adjustment of the solution to pH 4.7 using a pH meter was affected by titration with a 0.05M sodium acetate solution. The total volume of the solution was made up to 50ml adding distilled water. This results especially the same ionic strength and pH in the tissue solutions as in the case of standard solution. The two electrodes were inserted into the solution which was stirred until a constant millivolt age reading on the meter was obtained.

The millivolt age readings with the standard solutions were plotted on the rectangular axis against their fluoride concentrations on the logarithmic axis of the semi logarithmic paper. These curves so obtained were straight lines. These standard curves were used to translate the millivolt age readings of the selected tissues, (bone, muscle, scale, gill ash) solutions to concentrations of fluoride. Five samples for each tissue at different exposures were analyzed and further subject to statistical analysis. The data thus obtained were tabulated.

RESULTS:-

The fingerlings exposed to fluoride accumulated considerable amount of toxicant in different tissues of its body. The results after 8 days and 15 days of exposure to 33.48mg F/l was given in Table-1. The fluoride accumulated after 8 days and 15 days of exposure to 66.976 mg/l was given in Table-2. There is significant

accumulation of fluoride in gills and bone during 8 days of exposure as well as 15 days of exposure. The accumulation is comparatively high in higher

concentration. The fluoride accumulation is almost nil in muscle and less in bone.

Table – 1: Effect of sub-lethal concentration (33.488 mg/l) of fluoride in the selected tissues of *Labeo rohita* (Hamilton). The accumulated fluoride values are expressed as µg/gm ash weight.

Name of the Tissue	Control	Duration of Exposure	
		8 Days	15 Days
Gills	21.85 ± 1.05	51.04* ± 6.83	25.62* ± 9.81
Scales	31.66 ± 4.27	125.00* ± 31.18	71.31*
Muscle	4.84 ± 2.01	6.91* ± 4.43	4.69 ± 0.541
Bone	4.15 ± 0.764	4.34 ± 0.634	8.83* ± 4.16

Each value is mean ± S.D of four observations

* Significant at p 0.05 Level; t-test.

Table – 2: Effect of sub-lethal concentration (66.976 mg/l) of fluoride in the selected tissues of *Labeo rohita* (Hamilton). The accumulated fluoride values are expressed as µg/gm ash weight.

Name of the Tissue	Control	Duration of Exposure	
		8 Days	15 Days
Gills	21.85 ± 1.05	52.08* ± 9.08	56.26* ± 14.88
Scales	31.66 ± 4.27	141.66* ± 17.67	208.33* ± 17.67
Muscle	4.84 ± 2.01	4.832* ± 0.80	6.456 ± 2.05
Bone	4.15 ± 0.764	5.31* ± 0.54	13.12* ± 0.69

Each value is mean ± S.D of four observations

* Significant at p 0.05 Level; t-test.

DISCUSSION:-

Annie Thomas and Remya James (2013) studied about fluoride toxicity and accumulation in fresh water fish and reported that F accumulated even when present in low concentration in the medium. *Eetroplus suratensis* (Pearl spot), *Oreochromis mossambicus* and *Anabas testudineus* accumulated fluoride in varying amounts basing on the seasonal changes in the effluent surface fresh waters. The accumulation was more significant. The accumulation was more in *E. suratensis* and less in *O. mossambicus* followed by *A. testudineus*.

It was reported by XiaotaoShi, RuifangWang (2009) juvenile Siberian sturgeon (*Acipenser baerii*) when exposed to fluoride ion concentrations of 4mg F–L–T significant increase in F–L concentrations were observed in bone, cartilage, skin and gill.

In the recent studies XiaotaoShi, RuifangWang (2009) reported that the fish exposed to fluoride contaminated water accumulated more in gills, scales, bone followed by cartilage. Gills have the highest level of fluoride. Bio availability of F– is more in fresh water than hard water.

Hence, I have selected the study of fluoride accumulation in *Labeo rohita* (Hamilton) the fresh water fish.

Steven Fleiss (2011) reported that juveniles and small individuals are most susceptible to fluoride. An increase in temperature as well as decrease in water hardness increases the acute toxicity of fluoride. Garrec and Plebin (1984) reported about the accumulation of fluorine in earth worms living in contaminated soils and the accumulation was directly related to the total fluoride content of these soils.

JinlingCao LingtianXie (2012) reported the toxicity of fluoride to common carp (*Cyprinus Carpio*) chronically exposed to fluoride. There is strong linearity between the exposure concentrations and accumulation of fluoride. Gills had the highest level of fluoride among all the tissues.

Molina Agarwal (2017) reported the impact of fluoride on the fresh water teleost *Heteropneustes fossilis*. In aquatic habitats fish are the most sensitive organisms and

get affected even upon exposure to mild concentration of fluoride and teleost is very sensitive to fluoride.

The toxicity of fluoride in different tissues occurred at both lower and higher fluoride concentrations and increase with increasing period of exposure (Anand Kumar et.al., 2007) The results of the present study are also cognizance of the previous reports of Anand Kumar et.al (2007) JinglingCao, LingtianXie (2012) .

Hemens and Warwick (1975) reported fluoride accumulation in fish that resulted from fluoride in solution in water in the vicinity of effluent in Gabes Gulf. They conducted the experiments by exposing the mullets in water containing 52 ppm fluoride for 72 days and reported that the mullets accumulated 7743 ppm F/ashes, i.e., 520 ppm/wet weight whereas the control contained only 149 ppm/ashes.

Wright and Davidson (1975) reported that the fluoride accumulates primarily in the fish skeleton in the same way as the other vertebrates. Wright and Davidson, they reported the differences in the accumulation of fluoride are very little in the fish caught in the north Cambrian coast in which fluoride ranges from 1.2 to 1.5 ppm. Fluoride concentration in Cod “*godus morrhua*”, haddock. “*gaddus C aeglifinus*” and dab “*pleuronectis limanda*” vary between axial skeleton 18.2 at 99.7mg/kg wet weight, skin 13.3 at 74.3 mg/kg wet weight, muscle 0.05 at 3.7mg wet weight. IRPTCH 25(1978) reported that results of the Stewart et.al. in different species of fish taken in a non-polluted estuary at New Zealand the fluoride concentrations found from 509 to 28.85 ppm.

Tiews, Momthey and Koops (1982) reported that the rainbow trout when fed with krill meal pellets containing 2500mg F/kg for 90 days did not accumulate fluoride in the muscle tissue, but the deposit of F in the skeleton was considerable i.e., 3100mg/kg wet weight.

Mc Clurg (1984) reported that when estuarine prawn *Penaeus indicus* when exposed to fluoride and mercury. Mercury was deposited in varying degrees in the skeletal and non-skeletal tissues while the fluoride was confined almost to the skeletal tissues.

CONCLUSION:-

The defilement of water as fluoride contamination results in not only toxic action but also imbibed in

different tissues of the fish. When such fish are consumed by human beings the toxicants may be transported via the trophic link. Hence in the environmental policy and planning it should be monitored so that the toxicant is at minimal level. It requires further, study to understand the actual mechanism of accumulation of fluoride in the aquatic organisms.

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