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FLUORIDE TOXICITY IN FRESHWATER FISHES AND AQUACULTURE: A REVIEW

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ABSTRACT

The intake of fluoride taken place by inhalation, ingestion and absorption through the skin, damage the cellular structure & disrupts physiological activities, metabolic functions. Although, low concentration fluoride in drinking water are beneficial for normal tooth and bone development. But high concentration of fluoride in drinking water due to anthropogenic sources may causes impairment of metabolism in organisms including fish and its effect on fish health. Fish, are sensitive to environmental disturbances and in particular to any chemical change in aquatic environment. Fluoride mainly affects the level of glucose, lipid, protein, cholesterol and glycogen, all of which play an imperative role for growth, reproduction and survival of fishes. Alteration in levels of these bio-molecules can cause reduction in fish growth and population & reproduction. Present article is a review of potential adverse effects of fluoride in fishes and thus reflect the role of F on aquaculture. Furthermore, the information may be useful in the environmental risk assessment of freshwater fish &other organisms due to fluoride.

KEYWORDS : Fluoride Toxicity, Aquatic Behaviour, Fresh Water Fish

Aquaculture involves culture of freshwater and saltwater fishes under controlled condition. Fish growth is affected by physicochemical environment such as temperature, light and water quality in general health problem of fishes. Freshwater fishes dominate global aquaculture production. Water quality is related to the physical, biological and chemical parameters which affect the growth and fecundity of cultured organisms. Aquatic environment can be polluted by a variety of pollutants that originate from natural and anthropogenic sources which are toxic to the aquatic organisms including fishes. Fluoride is one of them. Fluorides are properly defined as binary compounds or salts of fluorine. The fluoride minerals, fluoride-rich minerals in the rocks and soils getting their final way into the water bodies are the main cause of high fluoride contents in water bodies. Anthropogenic sources of fluoride are industries, cosmetics and various products of day to day use (Neuhold and Sigler, 1960).Biomass, total body length, RNA: DNA ratio and energy reserve (total lipid, total protein and total glycogen) are the biomarker for evaluating health condition and growth rate of aquatic organism.(Dalhof, 2004; Giesy and Graney, 1989; Miliou et al., 1998). Retarded growth, leads to lower fecundity as well as reduced overall population fitness (Calow and Sibly 1990; Attrill and Depledge, 1997).Thus can be regarded as a ecologically relevant endpoint in ecotoxicological studies.

Fluoride toxicity to aquatic organisms including fishes increases with increasing fluoride concentration, temperature, exposure time and decreases with increasing intraspecific body size and water content of calcium and chloride (Neuhold and Sigler, 1960). However in soft waters with low ionic content of fluoride concentration low as 0.5 mg F^{-}/ I can adversely affect invertebrate animals and fishes, below safe level fluoride concentration are suggested in order to protect freshwater animals from fluoride toxicity. The optimum concentrations are significantly erratic within classes, families, and genera (Camargo J. A., 2003). Freshwater invertebrates and fishes, mainly net-spinning caddisfly larvae and upstream-migrating adult salmons, to be more sensitive to fluoride toxicity than marine and estuarine animals (Camargo J. A., 2003). Fluoride is highly transportable inorganic pollutant and its availability may increase the permeability of cell membrane in such a way that it provides help in accumulation of essential and non-essential metals in the organs that are directly exposed to toxicants such as skin and gills. Furthermore any increase and decrease of the bioavailability of micronutrients metals adversely affect the physiological activities of various organs (Azmat et al., 2011). Neuhold and Sigler (1960) reported that habitat of trout with measurable level of fluoride induced delayed migration. In addition, research on the molecular mechanisms of fluoride toxicity indicates that certain xenobiotics can induce excessive production of free radicals and affect the antioxidant defence system. (Hassan et al., 2009; Liu et al., 2011). The intake of fluoride by ingestion

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and absorption via skin, damage the cellular structure which upshot in accumulation of other toxic metals in the body. The rate of absorption of oxygen through gills and skin depends on the availability or fluoride concentration. Higher the fluoride concentration lowers the absorption of oxygen which will be later on produced alteration in all biochemical processes occurring in fish. Shi et al., (2009 a, b) reported significant increase in fluoride concentration in bone, cartilage, skin and gill of Siberian sturgeon exposed to lethal dose of fluoride. Accumulation of Chemical in aquatic organisms, particularly fish which is consumed by human beings in large quantities are of special concern because a high retention of toxic substances in fish tissue may be detrimental to human health problem. The permanent pollution in aquatic environment leads to accumulation of pollutants in a fish. Accumulations of fluoride in the bone tissue reflect the contamination of the entire fish, (Begum et al., 2008). Shi et al., (2009b) has showed that in lab environment, accumulation of fluoride concentration 4mg/L leads to in juvenile Siberian sturgeon up to the level of 3204.4 mg F-/kg in bone, 1401.2 mg F-/kg in cartilage and 100.1 mg/kg in skin and 10mg/L in water being harmful to the growth of fish (Klein et al., 1974) .

National Cancer Institute Toxicological Program categorizes fluoride to be an equivocal carcinogen.On the basis of literature it suggests that fluoride is a most damaging environmental pollutant and is deliberated as mutagenic agent, genotoxic, and neurotoxic it may induce genotoxic, neurotoxic and mutagenic effects in aquatic fishs. (Mclvor, 1990; Bhatnagar et al., 2005; Azmat et al., 2007; Tripathi et al., 2009). Various studies showed that inorganic fluoride ions are directly toxic to aquatic habitat as well as terrestrial life and easily accumulate in the tissues especially aquatic fish, at higher concentration however absorption rates are greater than excretion rates due their perseverance of environmental factor and their propensity for bioaccumulation (Foulkes and Anderson, 1994; Masoud et al., 2006; Gosh and Adhikari, 2006).Various reports are available regarding the effect of fluoride on reproduction, growth as well as development of mammals (Chinoy et al., 1997). The frequent absorption of the fluoride may cause tooth decay, damage of bones, kidneys, muscles and nerves. The adverse effect of fluoride toxicity arises due to changes in several mechanism like as enzyme inhibition, collagen breaks down, gastric damage and disruption of the immune system (Manna et al, 2007). Generally most of the work has been carried out on the mammals and the human population inhabiting polluted industrial area. Very few reports are available in fishes. With reference to all the aspects, fishes are very important from nutritional point of view as well as economical venture as it is one of the main components of aquaculture.

Effect of Fluoride on Growth and Development

Study on growth of fish has received attention recently. It is an important aspect of modern fishery biology and essential requisite for aquaculture. During growth study physical variables are taken into consideration like as length, weight, volume etc. Exposures to trace elements and fluoride affect the developmental stages of aquatic organism (Thurberg et al., 1975;). Tripathi et al., (2005) found that the higher concentrations of Fluoride inhibits the growth of fishes such as weight, length and of fingerlings of *Hetropneustis fossilis*. Effect was more pronounced in fingerlings in comparison to young and mature fish. Ellis et al., (1948) reported that fish egg subjected to 1.5ppm fluoride had delayed hatching time. Sensitivity to fluoride is dependent on several factors such as size of the fish, temperature of the medium, calcium and chloride concentrations in the environmental medium. The sizes of fish also affect the fluoride toxicity level and accumulation rates. Larger fish are more tolerant in higher concentration of fluoride levels and accumulate less fluoride according to their body weight (Hemens et al., 1972). Higher than optimum concentration of calcium in the medium or the food, tend to amplify the resistance of fish to fluorides (Sigler, 1972). According to Bajpai et al., (2010) lipid and protein act as growth bioindicator against fluoride pollutant in *Hetropneustis fossilis*. These biomolecules depleted after the chronic exposure of fluoride, therefore it suggested that fluoride reduce the synthesis of biomolecules required for the appropriate growth and development of fish.

Effect of Fluoride on Chromatophores

The various studies indicating adverse effect of pollutants in fishes but very little information is present about their effect on pigmentation. Chromatophores are accountable for colour changes in fishes at the time of protection, courtship, mating and reproduction (Fujii, 2000). The study on effect of fluoride on coloration in *Hetropneutes fossilis* and *Channa punctatus* respectively found that persistent exposure of sodium fluoride causes the altered shape, size, dispersion quality of chromatephores in the skin of *Hetropneustes fossilis*. Chromatophore numbers increased while their size was reduced and shape of chromatophores become stellate in comparisons to reticulate chromatophore of controls.(Tripathi et al., 2005; Bajpai et al., 2012).

Effect of Fluoride on Behaviour

Behavioural alteration can be taken as sensitive indicators of environmental stress. Many workers have studied about behavioural changes in aquatic organism caused by pollutants and concluded that all behavioural changes associated with metabolic (Shaikh, 1999). Fluoride induces change in behaviour of fresh water fishes reported from different studies. (Aziz et al., 2014). Narwaria et al., (2012) recorded as the behavioural responses including habit, body position, opercular movement, food sensitivity and swimming movements induced by sodium fluoride. However the accumulation and increased secretion of mucus in the fish exposed to fluoride may be an adaptive and protective response to avoid the absorption of the applied toxicant by the overall body surface. (Das and Mukherjee, 2003; Yilmaz et al., 2004). Bajpai et al., (2009) have also reported that behavioural abnormalities such as fast breathing, loss of schooling behaviour, erratic swimming and secretion of large amount of mucus on body of *Heteropneustes fossilis* during exposure to sodium fluoride in his experimental study.

Effect of Fluoride on Haematology

Kamble and Vellal, (2010) reported that dose of fluoride concentration 100ppm, 200pm and 300ppm caused time dependent and dose dependent transient effect on RBC, WBC count and Hb, which indicates immunological suppression. According to Kumar et al., (2010) fluoride causes significant decreasement in content of red blood corpuscles (RBCs) , hemoglobin content (Hb) and packed cell volume (PCV) and oxygen carrying capacity of blood in *Clarias batrachus*. Guru et al., (2014) and Saksena et al., (2001, 2002) also reported that TEC, Hb, PCV, MCV, MCH and MCHC progressively decreased with an increase in the concentration of fluoride in *Channa punctatus*. Gupta et al., (2002) also found that fluoride decreases TEC, Hb , PCV, ESR while increase TLC in *Channa punctatus* and *Lobeo rohita*.

Effect of Fluoride on Biomolecules

Earlier reports that fluoride can induce many biochemical changes in mammals including rats, rabbits, goats and human being (Shashi et al., 1989 and Chinoy et al., 1994) but very few studies are available in fishes. Chitra et al., 1983 and Kumar et al., 2007 observed that fluoride affects the certain biomolecules and enzymes in different tissue of fresh water fishes. Fluoride mainly affects the level of glucose, lipid, protein, cholesterol and glycogen, all of which play an important role for growth, reproduction and survival of fishes. Alteration in levels of these biomolecules can cause reduction in fish growth and population. Calcium and magnesium act as second messenger for replication transcription and translation. Fluoride reduces the absorption of both from gut (Verma et al., 2002 and Machoy, 1995). Dausset et al., (1987); Gikunju et. al, (1992) have reported that fluoride increase the cholesterol level in liver, muscle, testis of fishes.

Aziz et al., 2013 have found that fluoride increased alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) level in gills of fresh water fish *Tilapia mossambica*. Accordingly to increased level of these three biomarkers enzyme are due to disturbances in carbohydrate and protein metabolism. The exposure of the larvivorous fish *Poecilia reticulata* to sodium fluoride for a period of sixty days revealed stress on respiratory metabolism in the animal and it lead to decreases Succinic dehydrogenase and Lactate dehydrogenase (LDH) enzymes involved in carbohydrate metabolism. The decrease in concentrations of both enzymes, Succinic dehydrogenase and Lactate dehydrogenase might have lead to metabolic shift from aerobic to anaerobic mode of respiration during the toxic phase of fluoride induced stress (Shingadia et. al., 2013). Panchal et al., (2014) and Tao et al., (2005) also reported that fluoride disturbed the activities of malondialdehyde (MDA), Superoxide dismutase (SOD), Gluthione Peroxidase (GSH-Px),Catalase (CAT), Glutathione Transferase (GST) and Xanthine Oxidase (XOD) in rat and in growing pig.

Nucleic acid (RNA/DNA) is biochemical biomarkers for evaluating the consequences of environmental change. Fluctuation in RNA/DNA or protein synthesis in response to changes environment can provide estimate of growth rate and general health circumstances in a rapidly changing natural environment (Dahlhoff, 2004). (Yang et. al., (2002); Ibiam and Grant, 2005; Fonseca, (2009) studied the effect of Cd, Pb, Zn, Cu, 2, 4-dichlorophenol (2,4-DCP) in *Caenorhabditis elegans* (Roundworm) *Solea senegalensis* (Senegal sole), *Skeletonema costatum* (Diatom), respectively and found that there was dose dependent reduce in RNA/DNA ratio. Freshwater fish *Channa striata* exposed to sub lethal concentration of cypermethrin showed significant decreased in the level of DNA, RNA and ,While the RNA/DNA ratio significantly changed at different exposure periods as compared to control group (Raksheskar and Gracy, 2012). Some research showed that fluoride lowered the RNA, DNA concentration in liver, brain, ovary of vertibrates (Verma et al., 2007; Jha, 2012). There is no any research which showed the fluoride effect on DNA, RNA content and RNA/DNA ratio in aquatic organism.

Effect of Fluoride on Reproductive System

Several investigations on animals suggest that fluoride reduced the fertility in male (Chinoy et al., 1997; Ghosh et al., 2002). Fluoride adversely affects the structure and mobility of sperm causes alteration in the level of reproductive hormones. Fluoride has been reported to increase the level of FSH and LH (Tokar and Savchenko 1977; Ortiz et al., 2003) , decreases the level of estrogens and testosterone,(Chinoy et al., 1992; Jiang et al., 2005) and disturb the ratio of estrogen receptor to androgen receptor ratio (Long et al., 2009). Wan et al., (2006) found that NaF caused disorganization, denudation, and reduction in germinal epithelial cells of the seminiferous tubules and an accompanying absence of sperm in the lumina in histological segment. A decline in sperm viability and a significant increase in sperm abnormality were also observed by Wan et al., (2006). Pati and Bhunya, (1987) reported that NaF causes cytogenetic changes in bone marrow and sperm cells viz. Micronuclei, chromosomal aberrations and alterations in sperm morphology respectively. According to Dominok and Miller, (1990), NaF induces mutations in the germ cells of male fruitfly (*Drosophila melanogaster*). Study of Kumar and Susheela, (1995) and kumar et al., (2010), in rabbit suggest that fluoride decreases epithelial cell height, increased dialation of the tubule, damage of stereocilia and secretory granules in the caput and cauda ductus epididymis in rabbits. These changes, it is proposed, adversely affected the structure and maturation of spermatozoa passing through the ducts. The weights of the caput epididymis and cauda epididymis also significantly reduced. Recently it has been reported that fluoride damaged the uterine cells in albino rat with increase level of fluoride (Kumari M., et al., 2011)

All above mentioned research has been carried out in human beings and mammals, which suggest that fluorides interfere with the reproduction, but a few reports are available in fishes regarding the effect of fluoride on reproduction .Fishes having good nutritive value and major source of protein for masses. It is well known that fluoride act as xenobiotic and xenobiotics in water affect the proper growth, differentiation of organs and other reproductive stages in fishes. (Jalabert et al., 2000). Kashirsagar et. al (2011) reported that fluoride induces the disorganization of ooplasm , inhibition of ovarian development , damaged oocyte and empty space of follicle in fresh water fish *Rita rita*. Hitesh .u. shinadia, (2001) found that fluoride causes histoarchitectural changes in testis and ovary of larvivorous fish . In testis it causes degeneration of seminiferous tubules and their epithelium due to denudation and vacuolization of cells, atrophy of spermatocyte and hyperplasia of sertoli cells.in ovary it causes hyperplasia of germinal epithelial and involution of ova, decresed frequency frequency of oocyte maturatin , cytoplasmic vaculolation. Kumar et. al., (2007) found that total protein and lipid in testis of

Hetropneustis fossilis, while cholesterol increase after exposure but glycogen decreases at lower concentration but increased in higher concentration.

Genotoxicity and Cytotoxicity of Fluoride in fish

An exposure to high fluoride concentration has been reported that it inhibit the cell proliferation, growth and induces apoptosis. Few research data suggest that fluoride influences different singling pathway involved in cell proliferation and apoptosis (Barbier et al., 2010). Cytotoxic and genotoxic studies in fish are demonstrating that sensitivity of these organisms. Micronuclei test (MNT), Chromosomal aberration test (CAT), Comet assay are the most widely used method to prove genotoxicity in fish (Garg et al., 2012). Some reports indicate that fluoride causes chromosomal aberration, DNA damage in mammalian cells. (Joseph et al., 2000 ; Podder et al., 2008). Tripathi et al., (2009) found that chromosomal aberration increased with dose dependent manner in *Clarias batrachus* after the exposure of fluoride. Jha, (2004) reported that DNA and cytogenetic alterations in aquatic organisms impaired enzyme function or general metabolism, abnormal development, cytotoxicity, immunotoxicity, reduced survival, growth and reproduction potency .

Effect of Fluoride on Histopathology

Kumar et al (2010) and Bajpai et al., (2012) found that when fish exposed to Fluoride , primary and secondary lammelar epithelium become swelled and clubbing on the tip of secondary lamellae of gills, shortening, and fusion of secondary lamellae, hyperplasia and hypertrophy in chloride cells of gills. According to Haque et al., (2012) fluoride causes histopathological alteration in soft organs (stomach, intestine, liver, kidney) of *Channa punctatus*. In stomach it causes disarrangement of mucosal fold, degeneration of epithelial cell. In intestine it induces degeneration in villi and necrosis in absorptive columnar epithelial cells. In liver, it causes necrosis in centrolobular area and degeneration in cytoplasm of hepatocyte. In kidney, disruption of bowmen capsule and detriration in the epithelial cell lining of proximal tubule. Chhaya et al., (2007) also reported same histopathological alteration in gill, kidney, intestine of fresh water fish, *Lobeo rohita*. Yadav et al., (2014) found that fluoride causes vacuolization, pyknotic nuclei , disruption an rupture as well as hypertrophy and hyperplasia of hepatocyte of *Hetropneustes fossilis*. Kashirsagar et. al., (2011) and Hitesh. U. shinadia, (2001) reported that fluoride induces histopathological alteration in ovary and testis of freshwater fish *Rita rita* which we have already mentioned in above heading. Tripathi et al., (2006) reported the effect of fluoride on vertebral coloumn of *Channa punctatus*. His observation revealed that fluoride causes decreasement in diameter of neural canal and inceasement of bone density.

DISCUSSION

All above mentioned research indicate that fluoride causes the growth retardation, behavioural changes, biochemical changes, reproductive infertility in vertebrates and in invertebrates. Fluoride reduced the number of total and alive of newborns per surviving adult after short- term exposure respect to control. The number of embryos reduced by the highest fluoride concentration. The behavioural activity (e.g. time to start normal movement) was affected by the highest fluoride concentration during the long-term bioassay. Fluoride is toxic at short and long-term exposures. At short- term causing mortality and at long- term affecting growth, reproduction and behaviour (Alonso and Camargo, 2010). All parameter affected by fluoride are interlinked to each-other i.e. long exposure of fluoride to fishes causes retardation of growth, mortality , availability and disease in fishes. Therefore human health affected to fluoride by directly and indirectly. Some research works have been carried in out our lab in fishes to observe fluoride toxicity. Aquaculture production is vulnerable to adverse impacts of disease and environmental pollutants.

However, the literature regarding the effects of F on aquaculture species is relatively limited.

Therefore, we need more experiments and data for checking the fluoride toxicity in fishes and aquaculture. The future intensive investigations are required to identify the whole cascade of events involved in the development of fluoride-induced toxicity, as well as the mechanisms of their regulation. This will be useful in accessing the ecotoxicological significance of fluoride in aquaculture.

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