Cytogenetics is the study of morphology and behaviour of chromosomes during meiosis and mitosis. Karyomorphological analyses give the basic information about the chromosome number, morphology, sex chromosomes and karyotypic formula. Karyological information of about 746 species of fishes belonging to 147 families of 46 orders has been given. The chromosome number varies from 2n= 18 to 190. Family Cyprinidae includes 3000 species under 370 genera and forms the largest group. Out of these, 106 species have been cytogenetically investigated. Among these, 103 species possess 2n=50, which is considered as type number of the family Cyprindae (Nagpure *et al.*, 2016).

Muller (1927) started genetic toxicology and detect the effect of radiation on the genome. After 20 years, Auerbach and Robson (1946) recorded the mutagenicity caused by the effect of toxic chemicals. Cattanach (1966) also examined the genetic changes in animals induced by radiations and chemicals. Genotoxicity in fishes caused by various chemicals has also been studied by the various workers and their observations are based on following parameters.

- Behavioural responses and morphological changes
- Chromosomal analysis
- Micronuclei and Erythrocyte aberrations
- Histopathology in Gills, Kidney and Liver

Behavioural responses and morphological changes

Behaviour is considered as promising tool in ecotoxicology. Behavioural responses and morphological changes are diagnostic end points for screening and differentiating chemicals based on their mode of action and its effect on the health of fishes (Drummond *et al*., 1986). These changes allow an organism to adjust to external and internal stimuli in order to meet the challenges of survival in a changing environment. It is not a random process, but rather a sequence of activities ensures fitness and survival of the individual. Behavioural responses due to the exposure of various chemicals can be easily measured in fishes. Fishes act as bioindicators because they are very sensitive

to the changes occur in the aquatic environment and play an important role in monitoring the water pollution. Thus, behaviour is a selective response that constantly adapting through direct interaction with physical, chemical, social and physiological aspects of environment.

Warner *et al*. (1966) were the first to study behaviour of fishes exposed to toxaphene and observed the changes in movement and avoidance behaviour. Khangarot and Rajbanshi (1979) examined the abnormal behavioural changes like loss of equilibrium, irregular gill movements and absence of shoaling behaviour in *Rasbora daniconius* exposed to zinc. Similarly, Durve *et al*. (1980) monitored appearance of copious mucus on the skin, shrinkage of the gill lamellae and haemorrhages near the mouth and caudal regions at the time of death in the same fish exposed to zinc.

Marked behavioural changes were recorded by Kaur and Bajwa (1987) in fingerlings of *Cyprinus carpio* treated with zinc and cadmium. They observed restlessness, jerky movements, loss of balance, rapid breathing and stretched fins. Saglio *et al.* (1996) concluded that carbofuran produced severe behavioural alterations like swimming impairment, enhanced intra specific aggressiveness and decreased responses to chemical food stimuli in goldfish. Schmidt-Posthaus *et al.* (2001) observed mild ulceration of the upper and lower jaw in *Salmo trutta* and *Oncorhynchus mykiss* collected from polluted water.

Rahman *et al.* (2002) studied the effect of diazinon 60 EC on behaviour of *Anabas testudineus, Channa punctatus* and *Barbodes gonionotus*. Fishes showed restlessness, arena movements, loss of equilibrium, swimming on the back (at higher doses), increased opercular activities, strong spasm, paralysis and sudden quick movements during the exposure. The treated fishes became very weak, settled at the bottom and died in the higher doses.

Jayakumar and Paul (2006) reported the toxic stress on the fish, *Clarias batrachus* exposed to cadmium chloride. The fishes manifested the behaviour changes like restlessness, erratic swimming movements, increased ventilator and gulping activity. Omitoyin *et al.* (2006) and Adedeji *et al.* (2008) recorded the change in skin colour from normal to dark pigmented, particularly on dorsal

and lateral parts in *Clarias gariepinus* exposed to lindane and diazinon, respectively.

Kumar *et al.* (2007) evaluated the acute toxicity of cypermethrin and *k*-cyhalothrin in *Channa punctatus*. They observed alterations in behaviour like hyperactivity, loss of balance, rapid swimming, increased surfacing activity, enhanced rate of opercular activity and convulsions. They concluded that changes were more pronounced with k-cyhalothrin than cypermethrin. Pandey *et al.* (2009) examined effect of temperature and dimethoate in freshwater catfish, *Heteropneustes fossilis.* Fishes showed uncoordinated movements, erratic and jerky swimming, jumping out of water, decreased opercular movements, copious mucous secretions all over the body, surfacing and gulping of air in higher concentration. They also noted that fish mortality was increased with the increase in the temperature.

Halappa and David (2009) observed behavioural responses in freshwater fish, *Cyprinus carpio* due to the effect of chlorpyrifos. Fishes exhibited disrupted schooling behaviour, localization at the bottom, independence (spread out) in swimming followed by loss of coordination, irregular, erratic and darting swimming movements. Marigoudar *et al.* (2009) estimated the impact of lethal 4.0 µg l/g for (1, 2, 3 and 4 days) and sublethal concentrations 0.57 µg l/g for (1, 5, 10 and 15 days) of cypermethrin on behavioural responses in a freshwater fish, *Labeo rohita*. In lethal concentration, fishes immediately migrated to the bottom of tank and showed changes in behaviour like schooling behaviour, irregular, erratic and darting movements, imbalanced swimming activity, surfacing phenomenon, respiratory disruption, loss of equilibrium and fishes eventually died. On the other hand, in sublethal concentration, schooling behaviour and increase in ventilation rate was recorded, while after 15 days, fishes exhibited free, normal swimming and active feeding.

Wasu *et al.* (2009) evaluated the sublethal and chronic effect of carbaryl and malathion on *Clarias batrachus.* Morphological and behavioural changes like increase in opercular movements, bottom to upward movement to overcome hypoxic condition were seen after 48h and 96h. Other changes like

resting at bottom, excess secretion of mucus and colour change were also observed, while the loss of equilibrium was monitored only with malathion.

Dube and Hosetti (2010) examined the behavioural responses in the freshwater fish, *Labeo rohita* exposed to one third (106 µg/L) and one fifth (64 µg/L) of the LC_{50} value of sodium cyanide for 1, 5, 10 and 15 days. Fishes exhibited irregular, erratic, darting movements followed by hyper excitability, loss of balance and finally settled at the bottom. They slowly became lethargic, restless and secreted excess mucous all over the body.

Srivastava *et al.* (2010) recorded the behavioural responses in *Heteropneustes fossilis* exposed to dimethoate. They observed increase in opercular movements, abnormal swimming, loss of orientation, fading of body colour, tendency of muscular tetany prior to death, mucous secretion, which made fishes sluggish and lethargic.

Susan and Sobha (2010) determined the acute toxicity, oxygen consumption and behavioural changes in the major carps, *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* exposed to fenvalerate. They observed hyper excitation, loss of equilibrium, increased cough rate, flaring of gills, increased mucous secretions from gills, darting movements, hitting against the wall, curvature of spine and body acquired dark colour before death.

Yaji *et al*. (2011) evaluated the effect of cypermethrin on the behaviour and biochemical indices of freshwater fish, *Oreochromis niloticus*. Fishes exhibited loss of equilibrium, hyperactivity and increased aggression at the initial exposure periods (12h to 24h), while fishes were reactive to startle response after longer periods of exposure (more than 24 hours). They also detected that rate of tail fin movement gradually increased with increase in concentration from 24 to 96h followed by decrease in opercular movements.

Ezeonyejiaku *et al.* (2011) monitored toxicity and behavioural responses in *Oreochromis niloticus* and *Clarias gariepinus* exposed to copper sulphate. Fishes showed unsteady swimming pattern, loss of balance, remained in water suspended in vertical position with mouth up near water surface and tail pointing downward followed by sinking at the bottom on death. They

concluded that copper sulphate was found to be more toxic for *Oreochromis niloticus* than *Clarias gariepinus.*

Kaushal and Mishra (2011) performed comparative study of cadmium compounds on morphological and behavioural aspects in a fresh water fish, *Channa punctatus.* Fishes were exposed to cadmium chloride (620.09±3.7ppm), cadmium sulphate (695.10±2.0ppm) and cadmium nitrate (943.51±4.8ppm) based on their LC_{10}-LC_{90} values for 24h. The behavioural changes observed were jumping out of aquarium, increased swimming, restlessness, hyperactivity, lethargy, loss of equilibrium and hyperactivity at the time of death transient. Morphological changes recorded were lesions, discoloration of skin and chemical deposition on skin. They concluded that both behavioural and morphological changes were more pronounced in cadmium chloride than cadmium sulphate and less in cadmium nitrate.

Kaur and Dua (2012) studied behavioural and morphological alterations in Indian Carp, *Labeo rohita*, exposed to five municipal wastewater concentrations (100%, 50%, 25%, 12.5% and 6.25%) of Tung Dhab Drain, Punjab for 96h. Treated fishes of different wastewater concentrations and time durations showed increased gulping of air and surfacing in the beginning of experiment. At highest concentrations (100% and 50%), fishes showed sinking and rising, whirling, hyperactive, side swimming and hung vertically in tank prior to death, whereas at three lowest concentrations (25%, 12.5% and 6.25%), fishes exhibited head up swimming then stationary at the bottom. The morphological changes observed were dark body colouration, bulging of eyeballs, excessive mucus secretions, loosening of scales, haemorrhages on skin and fins, lateral flexure in caudal region and altered posturing of pectoral fins. They recorded that both the changes showed concentration dependent response.

Ganeshwade *et al*. (2012) detected the toxicity of endosulphan (96h LC_{50} value 0.0035 ppm) on a freshwater fish, *Channa striatus.* Fishes exhibited various behavioural and morphological changes like irritation to pesticides,

excessive mucous secretions, loss of equilibrium, very fast operculum movements, violent action of pelvic fins and spreading of the fins.

Kaushal and Mishra (2013) investigated the acute toxicity of cadmium compounds (cadmium chloride 561.11 µg/l, cadmium sulphate 463.32 µg/l, and cadmium nitrate 711.05 µg/l) based on their 96h LC_{50} values on a fish *Channa punctatus* for 24h, 48h, 72h and 96h. Fishes showed a marked change in their behaviour like jumping out of aquarium, increased swimming, restlessness, surfacing and hyperactivity. The morphological changes included discoloration of skin, deposition of thin layer of chemical deposition on skin, mucous secretions and scale depletion. The intensity of toxicity was maximum in cadmium chloride than in cadmium sulphate and least in cadmium nitrate.

Walia *et al.* (2013b) reported various behavioural and morphological changes in a fish, *Labeo rohita* exposed to three sublethal concentrations (3.53%, 1.76% and 0.88% based on 1/2, 1/4, 1/8 of 96h LC_{50}) of tannery industrial effluent. They observed behavioural changes like erratic swimming, gulping of air, opercular movement, loss of equilibrium, restlessness and sluggishness, whereas morphological changes like loosening of scales, sinking of eyeball, redness of eye, profuse mucous secretions, bleeding from gills and haemorrhages were recorded. They concluded that 3.53% concentration proved to be more toxic than 1.76% and 0.88% concentrations.

Kaur *et al*. (2013b) studied behavioural and morphological changes induced in *Cirrhinus mrigala* exposed to three sublethal concentrations (24.48%, 12.24% and 6.12%) of dyeing industrial effluent. The various behavioural responses like erratic movements, gulping air on the surface or jumping out of water, opercular movements, loss of equilibrium, hitting against the wall, restlessness, sluggishness, fishes lied on the water surface before death, while morphological changes like loosening of scales, redness in eyes, profuse mucous secretion, bleeding from gills, ballooning and belly upward, pigmented patches on the abdomen were observed. They recorded that these changes were more at higher concentration (24.48%) and duration of time (96h) as compared to other concentrations and time durations.

Aziz *et al.* (2014) studied the behavioural and morphological alterations in a fresh water fish, *Tilapia mossambica* from Keenjhar Lake, Pakistan under the effect of sublethal concentrations of flouride (1.5g/70 L and 3.0 g/70L NaF) for 7, 14, 21 and 28 days. After 7 and 14 days, fishes showed marked apathy, restlessness, increased mucus secretion, gulping of air, reduction of swimming ability, darkening of color and loss of orientation equilibrium. Finally, there was reduction of swimming ability, darkening of color then death after long exposure.

Sultana *et al*. (2014) determined the impact of sublethal concentrations (75, 150, 300 ppm) of kerosene in a freshwater fish, *Channa marulius.* They observed morphological changes like appearance of black spots over the body surface, loss of scales, skin become dull, liberation of large amount of mucous and excreta, whereas behavioural changes included erratic movements of the operculum, resting flat at the bottom of the aquarium, drifting up and down frequently with widely opened mouth. The effect of toxicant was concentration and time dependent.

Kaur and Dua (2015) examined the various behavioural and morphological alterations in a freshwater fish, *Channa punctatus* due to wastewater toxicity from site 1 (near a paper mill) and site 2 (village Mahal of Tung Dhab drain) in the state of Punjab. LC_{50} of the wastewater of both sites was calculated and five concentrations (100%, 50%, 25%, 12.5% and 6.25 %) of each sample were selected. Various behavioural changes like increased air gulping, surfacing, decrease in number of opercular movements, erratic movements, loss of equilibrium, hyperactivity and mouth pointed towards the surface prior to death were observed. Morphological changes included sinking of eyeballs, copious mucus secretions, increased body coloration, haemorrhages and loss of scale. They concluded that the effect was more pronounced in site 1 than site 2 and the changes showed time and concentration dependent response.

Brraich and Kaur (2015) studied behavioural and morphological manifestations in *Labeo Rohita* due to the effect of sublethal concentrations

(3.42mg/l, 4.84mg/l, 6.88mg/l and 11.4mg/l) of lead nitrate. They observed behavioural changes like restlessness, swimming impairments, jerky movements, loss of equilibrium, drowning, hitting against the wall of test aquaria, increased and fast opercular movements, rapid surfacing and gulping of air and severe diarrhea, while morphological anomalies were shedding of scales, fusion of fins, lesions on skin, eye deformities, muscular tetany, caudal bending/scoliosis, dullness in the body colour along with profuse mucus secretions. The alterations in the behaviour and morphology showed concentration and time dependent responses.

Walia and Handa (2016) observed behavioural changes in fish, *Labeo rohita* exposed to two sublethal concentrations (7.74% and 1.93%) of tannery effluent. Fishes showed erratic swimming, gulping air at surface and hitting against the wall from 24h to 120h, fast opercular movements upto 48h and slow down after 72h to 120h. Fishes lost their equilibrium and were sluggish from 72h to 120h. Two fishes died at 120h at higher concentration. In low concentration, the effect was less severe. Fishes possessed erratic swimming, gulping of air at surface, slow opercular movement from 72h to 120h.The fishes started hitting against wall from 96h to 120h. They fishes became sluggish after 120h. The alterations revealed concentration and time dependent response.

Walia and Kalotra (2016) observed morphological changes in *Cirrhinus mrigala* exposed two sublethal concentrations (26.24 % and 6.56%) of dyeing industry effluent. They observed morphological changes like loosening of scales, redness in eyes, profuse mucous secretion, bleeding from gills, ballooning and pigmented patches on the abdomen. In both the concentrations, loosening of scales was present from 96h to 120h, whereas profuse mucous secretions and pigmented patches on the abdomen were present from 72h to 120h. In lower concentration, redness in eyes and ballooning were absent, whereas in higher concentration these were present from 72h to 120h.

Chromosomal analysis:

Genotoxic studies using cytogenetic analyses in fishes have explained the sensitivity of these organisms (Al-Sabti and Metcalfe, 1995). Genotoxicity describes the property of chemical agents that damages the genetic information within a cell by mutations, which may have direct or indirect effects on the DNA. Numerous toxic and genotoxic contaminants, such as trace metals, pesticides, dyes and pharmaceuticals are proved to disturb the aquatic environment. Aquatic ecosystem is a best option to monitor the genotoxicity of inorganic as well as organic compounds, because the pollutants (genotoxicants/genotoxins) present in air and soil are ultimately, enter in the water. It is well known that the disposal of pollutants into aquatic ecosystems can lead to their accumulation in sediments, benthic and pelagic food chains (including fish). Fishes are best experimental model organism as they are easy to handle, inexpensive, require limited amounts of space and equipment. Some genotoxic effects are irreversible, thus, analysis of environmental genotoxicity is need of the hour to evaluate the long term effects of contaminants. Genotoxic studies based on chromosomal aberration test in different species of fishes have been reported by various workers.

Hooftman (1981) observed chromosomal aberrations in *Notobranchius rachowi* treated with ethyl methane sulphonate or Benzo (a) Pyrene. Al-Sabti (1985) studied the chromosomal aberrations in rainbow trout, *Salmo gairdneri* exposed to five pollutants. He concluded that neguvon and malathion increased nonspecific aberrations, while phenol enhanced the percentage of aneuploidy. Further, malathion also showed structural aberrations like breaks, fragments, chromatid gaps, ring and dicentric chromosomes. Krishnaja and Rege (1982) recorded chromosomal aberrations like chromatid breaks, gaps, rings and fragments in *Bleophthalmus dussumieri* exposed to 30.5 ppm chromium for 96h. Al-Sabti and Kurelec (1985) examined chromosomal aberrations in *Mytilus galloprovincialis* collected from ten different sites at the rovinj area of Northern Adriactic Sea. They found chromosome breaks and fragments in the

fishes of all the sites, while maximum number of cells with aberrations was present in the polluted water.

Matter *et al.* (1992) studied genotoxic effect of carbamyl insecticide on the grass carp, *Ctenopharygodan idella*. Rishi and Grewal (1995a) recorded various types of chromosomal aberrations like centromeric gaps, chromatid gaps, subchromatid breaks, attenuation, extra fragments, pyknosis, precocious separation and stubbed arms in *Channa punctatus* exposed to dichlorvis insecticide. Subsequently, Rishi and Grewal (1995b) screened kidney cells treated with the mosquito larvicide and observed similar chromosomal aberrations in *Channa punctatus*. Hayashi *et al.* (1998) evaluated the structural chromosomal aberrations in the embryonic cells of *Rhodeus ocellatus ocellatus* grown in the water containing trichloroethylene in the laboratory. The frequencies of aberrated cells were increased in fishes treated with mitomycin-C and in high dose of trichloroethylene. The results were also showing dose dependent response.

Visoottiviseth *et al.* (1998) examined the effect of triphenyltin hydroxide (TPTH) on the chromosomes of catfish (hybrid of *Clarias macrocephalus* and *Clarias gariepinus*). They observed normal chromosome complement in the control (water) and control (solvent) [dimethylsulphoxide (DMSO)], while nonspecific type of chromosomal aberrations found in 18% and 23% of the total cells and chromosomal deletions in 5.3% and 4.7% of the cells in the treated groups. They also recorded that increase in the dose of TPTH did not cause any significant increase in the percentage of chromosomal abnormalities.

Mathew and Jahageerdar (1999) analysed effect of lead nitrate (0.012, 0.025 and 0.050 mg/l) in *Channa punctatus* for 96h, 120h and 144h. They observed chromosomal aberrations like breaks, fragments, dicentric and ring chromosomes in all the concentrations. Krishna and Gupta (2002) reported chromosomal aberrations like breaks, fragmentation, acentric and dicentric chromosomes in *Labeo rohita* exposed to two concentrations (1.5ppm and 3 ppm) of copper exposure.

Guha and Khuda-Bukhsh (2002) reported effect of ethyl methane sulphonate (EMS) on fish, *Oreochromis mossambicus*. They found chromosomal aberrations like chromatid break, ring, acentric fragment, pulverization, C-mitotic effect. They also measured the decrease in frequency of chromosome aberrations by adding ascorbic acid in distilled water as a medium to EMS-treated fishes. Subsequently, Guha and Khuda-Bukhsh (2003) investigated the ameliorating effect of β-carotene (BC) on ethyl methane sulphonate (EMS) induced genotoxicity in the same fish. They found that frequency of aberrations in the EMS-treated fishes was significantly higher than the distilled water (DW) treated control, which indicated clastogenic effect of EMS. On the other hand, treated fishes with BC (0.05%) produced slightly more chromosomal aberrations than the DW-treated control. They also noted that the chromosomal aberrations was considerably enhanced in EMS- treated fishes injected with alcohol (1%), while the percentage of chromosomal aberrations in the EMS + BC (0.05%)-treated series was found to be reduced as compared to that of EMS + 1% alcohol-treated fishes. All the three doses of BC (0.02, 0.05 and 0.1%) when applied in combination with 0.02% EMS, reduced chromosome aberrations to a considerable extent as compared to those treated with 0.2% EMS + 1% alcohol. However, BC (0.02%) showed maximum antagonistic effect.

Cestari *et al.* (2004) determined the genetic damage caused by lead in the neotropical fish, *Hoplias malabaricus* by comet assay and chromosomal aberrations. They found increase in chromatid gaps, breaks, chromosomal fragmentation, chromatin decondensation and pericentric inversions, while chromatid breaks were occurred significantly.

Chandra and Khuda-Bukhsh (2004) studied the effect of cadmium chloride ($CdCl_2$) and azadirachtin (Aza) on the chromosomes of *Oreochromis mossambicus* when injected individually. However, when both $CdCl_2$ and Aza were conjointly administered, the clastogenic effects appeared to be reduced, which was statistically significant. Ferraro *et al.* (2004) assessed the mutagenic effect of tributyltin (TBT) and inorganic lead (Pb II) on the fish *Hoplias*

malabaricus. They recorded only structural alterations like gaps and breaks in chromosomes, while numerical changes were absent, although, decondensation of chromatin on chromatid arms was seen.

Abdel-Wahhab *et al.* (2005) detected the efficacy of Egyptian montmorillonite (EM, a clay mineral) for the absorption of sterigmatocystin (Stg, a mycotoxinaflatoxin) on the nile tilapia fish, *Oreochromis niloticus*. Stg treated fishes showed the increased frequency of chromosomal aberrations like centromeric attenuation, gaps/breaks, deletions and fragmentation, while EM treated and control fishes did not possess any chromosomal aberrations. EM and Stg treated fishes were depicting significant decrease in the number of chromosomal aberrations, which indicated that EM inhibited the impact of genotoxicity caused by Stg.

Velmurugan *et al.* (2006) evaluated the genotoxic effect of lambda-cyhalothrin on fish, *Mystus gulio*. They observed chromosomal aberrations like chromatid breaks, acentric fragments, centromeric fusions, aneuploidy, condensation, sticky plates and ring chromosomes. Moreover, high frequencies of acentric and chromatid breaks (72h) followed by sticky plates (96h) were also noted.

Kumari and Ramkumaran (2006) reported chromosomal aberrations in fish, *Channa punctatus* from Hussainsagar lake, Hyderabad (Andhra Pradesh). They selected live fishes as test specimen from lake, while fishes from non-polluted water bodies (Himayatsagar and Osmansagar) as control. They recorded that about 20% fishes from lake were possessing normal chromosomal complement as control, whereas 65% fishes were showing chromosomal aberrations like chromatid gaps, fragments, sister-chromatid exchanges, dicentric chromosomes and ring chromosomes.

Yadav and Trivedi (2006) estimated the genotoxic potential of chromium (VI) on fish *Channa punctatus.* Fishes were selected as group I (non-treated controls); group II (positive controls, treated with an intra-muscular injection of mitomycin-C at 1 mg/kg body wt); group III exposed to sublethal concentration (7.689 mg/l of [Cr(VI)] dissolved in the water) for 24h,

48h, 72h, 96h and 168h of exposure. The marked chromosomal aberrations recorded were chromatid breaks, chromosome breaks, chromatid deletions, fragments, acentric fragments, ring and di-centric chromosomes along with chromatid and chromosome gaps in both group II and III. A significant increase in chromosomal aberrations was observed after 72h treated with [Cr(VI)].

Ramadan (2007) evaluated the genotoxic effect of butataf herbicide on fish nile tilapia, *Oreochromis niloticus.* The exposed fishes showed both structural and numerical aberrations. He observed chromosomal aberrations like chromosomal deletions, chromosomal fragments, chromosomal breaks and chromosomal stickiness, whereas numerical aberrations like hyper and hypopolyploidy. He also recorded concentration dependent response. These results were in accordance with Shaban (1999), who found frequency of aberrations increased with increase in dose of alfatoxin B_1 ($AF\beta_1$) in both *Oreochromis niloticus* and *Cyprinus carpio*.

Gadhia *et al.* (2008) assessed the *in vivo* exposure (anti-neoplastics; bleomycin, mitomycin-C and doxorubicin) in a fish, *Boleophthalmus dussumieri.* They showed dicentric, ring configuration and translocations after 4h of initial exposure, while breaks and inter changes were noted after 24h.

Mohamed *et al.* (2008) determined the capability of copper sulphate ($CuSO_4$) and lead acetate [$(CHCOO)_3Pb$] to induce chromosomal aberrations in fish, *Oreochromis niloticus*. They noted different types of chromosomal aberrations like chromatid deletions, chromatid breaks, gaps, fragments, stickiness, translocations, ring chromosomes and centromeric attenuations. Among these, chromatid deletions and fragments were predominant, while frequency of translocations was less. Moreover, stickiness was prominent only in lead acetate.

Radwan and Ghaly (2008) evaluated the antimutagenic effect of neem leaves extract against sodium arsenate in a freshwater fish, *Clarias lazera.* They observed chromosomal aberrations like chromatid gaps, deletions, breaks, fragments, end to end associations and dicentrics. They recorded that frequency

of chromosomal aberrations induced by neem extract was statistically non-significant as compared to control, while increased significantly by sodium arsenate. On the other hand, neem extract also reduced the frequency of chromosomal aberrations induced by sodium arsenate.

Ramsdorf *et al.* (2009) investigated the genotoxic effect of inorganic lead (PbII) in a fish, *Hoplias malabaricus*. They observed only centromeric gaps as significant chromosomal aberrations in treated groups as compared to control. Hafez (2009) evaluated the genotoxicity and cytotoxicity induced by pollutants present in the Abu-qir bay on a fish, *Mugil cephalus* collected from four different sites. He recorded chromosomal aberrations like stickiness, fragments, gaps, breaks and deletions, whereas formation of micronuclei, nuclear buds, binucleated cells, fragmented and apoptotic cells in the blood, kidney and gills of fishes collected from different locations of the bay were also seen. He concluded that location number 4, El-Tabia pumping station was proved to be highly polluted.

Malik *et al.* (2009) studied the effect of paper mill effluent on *Channa punctatus* after 24h, 48h, 72h and 96h. They observed chromosomal aberrations like centromeric gap, chromatid break, attenuation, acentric fragment, pyknosis, stickiness, stubbed arm, precocious separation and polyploidy. They concluded that chromosomal aberrations were decreasing with the increase in duration of exposure.

Tripathi *et al.* (2009) examined the genotoxicity induced by fluoride (F) in the Asian catfish, *Clarias batrachus.* They observed chromosomal aberrations like chromatid breaks, chromosome breaks, chromatid deletions, fragments, acentric fragments, ring and dicentric chromosomes. They found more chromosomal aberrations in fishes treated with mitomycin-C (positive control) as compared to control, whereas these were significant in the fluoride treated groups but less than the positive control.

Yadav and Trivedi (2009) monitored the rate of chromosomal aberrations in a fish *Channa punctatus,* after *in vivo* exposure to mercuric chloride (group III), arsenic trioxide and copper sulphate. They found

chromosomal aberrations like chromatid/chromosomal breaks, chromatid/chromosome gaps along with ring and dicentric chromosomes. The frequency of chromosomal aberrations was significantly high in treated group of mitomycin-C and heavy metals.

Mahmoud *et al.* (2010) reported chromosomal aberrations in *Oreochromis niloticus* and *Tilapia zillii* collected from locations (A and B) of upstream and (C and D) of downstream to the sewage of Shanawan canal in Egypt. They observed chromosomal aberrations like centromeric attenuation, chromatid breaks, chromatid gaps, chromatid deletions, centric fusion and fragmentation. They found high frequency of chromosomal aberrations in fishes from areas C and D of downstream to sewage (two fold) than the fishes from areas A and B of upstream to the sewage source. Moreover, frequency of chromosomal aberrations was high in *Oreochromis niloticus* as compared to *Tilapia zillii*.

Saxena and Chaudhari (2010) recorded chromosomal aberrations like chromatid break, fragment, gap, chromatid separation, deletion and ring chromosomes in *Channa punctatus* exposed to fenvalerate for 96h. They also recorded time dependent response of the test chemical. Obiakor *et al.* (2010) determined the genotoxic effect of polluted river Oyi for 10 and 28 days on a catfish *Clarias gariepinus.* They concluded that chromosomal aberrations like chromosome break, fragment, acentric fragment and ring chromosomes were increased with the increase in the duration of exposure. Rose *et al.* (2010) also examined the effect of polluted water from two locations Saidapet and Avadi of river, Coovum on a freshwater fish, *Hypophthalmicthys molitrix.* They observed chromosomal aberrations like reduction in chromosomal number, endo-reduplication, chromosome fragments, poorly stained chromosomes, ring chromosomes, rod chromosomes, chromosome deletion and dyad chromosomes.

Ansari *et al.* (2011) evaluated the cytogenetic and oxidative stress induced by cypermethrin on a freshwater fish, *Channa punctatus.* They found predominant occurrence of cytogenetic damage like chromosome and

chromatid breaks in treated fishes as compared to control. They observed significant increase in chromosomal aberrations in the positive control [treatment with ethyl methane sulphonate (EMS)]. They also detected the maximum rate of chromosomal aberrations in fishes exposed to EMS and cypermethrin.

El-Araby *et al.* (2011) examined the effect of atrazine on *Tilapia nilotica*. They found various chromosomal aberrations like polyploidy, aneuploidy, gaps, break, deletion, ring chromosome and centromeric attenuation. Parveen and Shadab (2012) found the increase in the frequency of chromosomal aberrations like gaps, breaks, dicentrics and rings due to the effect of cadmium chloride in *Channa punctatus* after 72h. Srivastava and Singh (2013a) monitored the effect of sublethal doses of trizole (1.1 1 mg/l and 2.23 mg/l) in a fish *Clarius batrachus.* They observed decreased mitotic index after 24h, 48h, 72h and 96h. Subsequently, Srivastava and Singh (2013b) detected the impact of sublethal doses of mancozeb (11.43mg/l and 22.87 mg/l) in a fish *Clarius batrachus.* They observed decreased mitotic index after 24h, 48h, 72h and 96h. Yadav *et al*. (2013) exposed *Cirrhinus mrigala* to butachlor and observed chromosomal aberrations like stickiness, clumping, end to end joining, break, gap and fragments upto 48h.

Walia *et al.* (2013c) studied chromosomal aberrations in a fish, *Labeo rohita* exposed to three sublethal concentrations 3.53%, 1.76% and 0.88% of the tannery industrial effluent for 24h, 48h, 72h and 96h. They observed ten types of chromosomal aberrations, among these, chromosome fragments, ring chromosomes, centromeric gaps and minutes were predominant, while pulverization and stretching were lowest in all the concentrations. They found concentration and time dependent response.

Kaur *et al.* (2013a) examined the chromosomal aberrations in *Cirrhinus mrigala* exposed to three sublethal concentrations 24.48%, 12.24% and 6.12% of dyeing industrial effluent for 24h, 48h, 72h and 96h. They found centromeric gaps, clumping and ring chromosomes as predominant aberrations in all the concentrations. They concluded that chromosomal aberrations showed

concentration and time dependent response. Mahboob *et al*. (2013) detected the concentration dependent response of mercuric chloride in *Clarias gariepinus* after 168h. They observed chromosomal aberrations like gaps, breaks, dicentrics and rings.

Tiji and Adeogun (2014) studied chromosomal aberrations like breaks, rings, acentric and dicentric chromosomes in *Clarias pachynema* from downstream and upstream locations of polluted Ogun River, Lagos, Nigeria. They found no aberrations in the fishes upstream from the discharge point.

Walia and Handa (2016) studied chromosomal aberration in fish, *Labeo rohita* exposed to two sublethal concentrations (7.74% and 1.93%) of tannery effluent. They found that in higher concentration, mean percentage of chromosomal aberrations increased from 24h to 120h, whereas they decreased from 24h to 120h in lower concentration. All these aberrations revealed clastogenic effect. The results showed concentration and time dependent response.

Walia and Kalotra (2016) recorded chromosomal aberration in two sublethal concentrations (26.24% and 6.56%) of dyeing industry effluent. They observed five types of aberrations like chromosomal fragments, ring chromosomes, terminal chromatid deletions, minutes and aneuploidy. In lower concentration, mean frequency decreased from 24h to 120h, whereas in higher concentration, it increased from 24h to 120h.

Micronuclei and erythrocyte aberrations

The micronucleus (MN) test is one of the most popular tests of environmental genotoxicity to detect the effect of environmental agents in aqueous media and also serves as an index of clastogenic and cytogenetic damage (Fenech *et al.*, 2003; Udroiu, 2006). The chromosome aberrations lead to micronucleus induction in the cell/cells. Micronuclei are the small, chromatin containing bodies arising from the chromosomal fragment or whole chromosome due to spindle dysfunction. These lagging elements are much smaller than the main nucleus, therefore, called as micronuclei (MN) and are even passed to the daughter cells. Although, erythrocyte abnormalities (any

change in nucleus and cell structure of erythrocytes) are also considered as indicators of genotoxicity. Micronuclei and erythrocytes abnormalities help to assess the status of water quality as well as health of a particular species and their potential risk after consumption (Talapatra and Banerjee, 2007). These abnormalities are simple, give fast results and easy to detect as compared to chromosomes, which are small in size. The frequency of micronuclei and other nuclear aberrations provide the information about the frequency of chromosomal damage and risk to human health arising due to the presence of genotoxic environmental contaminants in aquatic ecosystems. In the recent years, apart from MN formation, the formation of other cytoplasmic and nuclear alterations in piscine erythrocytes have also been used as possible indicators of genotoxicity (Cavas and Ergene-Gozukara, 2005a, b; Da- Silva Souza and Fontanetti, 2006; Ergene- Gozukara *et al*., 2007; Cavas and Konen, 2007) .

Howell (l891) and Jolly (1905) gave the concept of nuclear remnants in red cells showing pathological alterations and named them Howell-Jolly bodies. Later, Evans *et al*. (1959) followed by Schroeder (1970) renamed them as micronuclei. They considered micronuclei as indicator of genetic damage, which used for the various genotoxicity tests.

Schmid *et al.* (1971), Heddle (1973), Von Ledebur and Schmid (1976) reported that micronuclei were used to monitor the cytogenetic damage. They developed *in vivo* test for the identification of micronuclei. Micronucleus test was used as a fast method for testing genotoxic effects in mammals (Evans, 1976; Schroeder, 1970*;* Sutou, 1981, 1986; Heddle *et al*., 1983). Modified micronuclei test and clastogen induced micronuclei in peripheral blood erythrocytes were studied by MacGregor *et al.* (1980).

Hooftman and De Raat (1982) evaluated the induction of nuclear and micronuclei anomalies in peripheral blood erythrocytes in mud minnow, *Umbra pygmaea* for the first time exposed to ethyl ethane sulphonate. Longwell *et al*. (1983) used micronuclei test to study genotoxicity in fishes; windowpane flounder, *Scophthalmus aquosus*, Atlantic mackerel, *Scomber*

scombrus, winter flounder, *Pseudopleuronectes americanus*, mummichog, *Fundulus heteroclitus* and coho salmon, *Oncorhynchus kisutch* collected from the field. Al-sabti (1986) tested several chemicals (alphatoxins B1, arochlor, benzidine, benzo (a) pyrene and 20-methyl cholanthrene) for the induction of micronuclei in three cyprinds (*Cyprinus carpio*, *Tinca tinca*, and *Ctenopharyngodon idella*). Das and Nanda (1986) measured the increase in micronuclei frequencies in the erythrocytes of the common Indian catfish, *Heteropneustes fossilis* exposed to mitomycin-C and paper mill effluent. Manna and Biswas (1986) found micronuclei in four species of fishes treated with bacterium, *Pseudomonas aeruginosa*. Manna and Sadhukhan (1986) detected the micronuclei in gills and kidney cells of Tilapia fish, *Oreochromis mossambicus*. Sadhukhan and Manna (1989) observed the micronuclei in peripheral blood, gills and kidney cells of *Oreochromis mossambicus* treated with rogor-30E (insecticide).

Al-Sabti and Hardig (1990) investigated the induction of micronuclei in perches collected from the Baltic Sea in the vicinity of a pulp plant in Sweden and recorded the increase in micronuclei frequencies. Matter *et al.* (1992) studied the occurrence of micronuclei in *Ctenopharygodan idella* exposed to insecticide, carbamyl. Al-Sabti *et al*. (1994) detected micronuclei in *Carassius auratus gibelio* exposed to chromium for 7, 14, 21 days. They found the presence of micronuclei in all the days, while the effect was least on 21st day.

Al-Sabti (1995) evaluated the occurrence of micronuclei in trout hepatic cells (*in vitro*) treated with water pollutants (selenium, mercury, methyl mercury and their mixture) in the field and laboratory conditions. He found concentration dependent response. Subsequently, Al-Sabti and Metcalfe (1995) reviewed the clastogenic effect of chemicals and physical agents for the induction of micronuclei in teleost fishes cells. They also summarized the various techniques used for micronuclei analysis in fishes for the water monitoring.

Poongothai *et al*. (1996) investigated the induction of micronuclei in five different fishes collected from polluted sewage water and treated with

heavy metals. They found that frequency of micronuclei was statistically significant in both the groups. They concluded *Lepidocephalus* as highly sensitive fish among the five species.

Hayashi *et al.* (1998) used aquatic organisms as a tool to assess the genotoxicity in the field and laboratory. Micronuclei test was done in *Carassius* species and *Zacco platypus* collected from four areas (upstream and midstream of Tomio river) throughout the year and found more micronuclei in midstream than upstream. Micronuclei frequencies showed seasonal variations in gill cells and erythrocytes of *Leiognathus nuchalis* and *Ditrema temmincki,* which were higher in summer season.

Matsumoto and Colus (2000) studied the micronuclei in *Astyanax bimaculatus* fish exposed to two mutagenic drugs cyclophosphamide and vinblastine sulphate. They found no significant difference in the frequency of micronuclei in different doses of vinblastine sulphate, whereas they observed dose dependent response in cyclophosphamide. They concluded that cyclophosphamide is best drug to induce positive response in fishes.

Ayllon *et al.* (2000) determined the effect of conventional armament discharged in river Trubia for micronuclei induction in wild brown trout, *Salmo trutta*. They found significant increase in micronuclei counts in downstream area, where the old military factory was located as compared to upstream area of river.

Chandra and Khuda-Bukhsh (2001) assessed the genotoxicity caused by cadmium chloride in fish, *Oreochromis mossambicus* and also detected the efficiency of vitamin-C to reduce the aberrations. Gustavino *et al.* (2001) examined the induction of micronuclei in *Cyprinus carpio* exposed to X-rays and colchicine. They observed dose dependent response due to irradiation with X-rays, whereas high concentration of colchicine showed lethal effect and also resulted in slight increase in micronuclei.

Ateeq *et al*. (2002) investigated the effect of two herbicides 2, 4-dichlorophenoxyacetic acid (2,4-D) and 2-chloro-2, 6-diethyl-n- butoxymethyl acetanilide (butachlor) for micronuclei induction in a catfish, *Clarias*

batrachus. They founded time and dose dependent relationship with respect to rate of micronuclei and erythrocyte aberrations in both the herbicides. They observed echinocytes with diffused nuclear material, vacuoles and other nuclear abnormalities predominantly in 2, 4–D, whereas anisocytes and anisochromasia were pronounced in butachlor stress. They concluded that 2, 4-D proved to be more toxic than butachlor in terms of chromosomal damage as well as for cellular abnormalities.

Guha and Khuda-Bukhsh (2003) reported micronuclei and erythrocytes abnormalities in a fish, *Oreochromis mossambicus* treated with ethlylmethane sulphonate (EMS), with EMS + 1% alcohol and Distilled water (DW), β-carotene (BC) as controls. They observed that the number of micronuclei and abnormal nuclei were decreasing in the distilled water (DW) or β-carotene (BC) - treated fishes. However, when EMS- treated fishes were counter-injected with BC showed fewer micronuclei and nuclei abnormalities as compared to EMS + 1% alcohol, whereas BC- treated fishes possessed slightly more micronuclei than of EMS + 1% alcohol treated fishes.

Mallick and Khuda-Bukhsh (2003) determined the micronuclei and nuclear anomalies in fishes collected from river Hoogly-Matlah, which received metallic compounds from Sunderban estuaries and industrial effluents of Calcutta and Howrah industries. They selected three sampling sites; Canning, Kakdwip, Haldia and seven species of fishes, *Lates calcarifer, Liza parsia, Liza tade, Mugil cephalus, Rhinomugil corsula, Terapon jarbua* and *Scatophagus argus* for the study. They found high percentage of micronuclei in all the fishes from Haldia followed by Kakdwip and lowest in the Canning. They also recorded increase in occurrence of nuclear abnormalities in all the fishes from Canning followed by Kakdwip and Haldia, whereas *Scatophagus argus* showed opposite trend. They concluded that data showed increasing trend in micronuclei, while decreasing trend for nuclear abnormalities.

Bhunya and Sahoo (2004) administered an insecticide carbaryl in the peripheral blood erythrocytes of *Anabas testudineus*. They found linear

increase in the frequency of micronuclei and nuclear abnormalities in treated fishes as compared to control.

Chandra and Khuda-Bukhsh (2004) recorded the effect of cadmium chloride ($CdCl_2$) and azadirachtin (Aza) for micronuclei induction in *Oreochromis mossambicus.* They found gradual increase in micronuclei in both the chemicals injected individually. However, when both $CdCl_2$ and Aza were conjointly administered, the clastogenic effects appeared to be reduced to some extent, which was statistically significant. On the other hand, opposite effect was observed in case of nuclear abnormalities.

Abdel-Wahhab *et al.* (2005) determined the efficacy of Egyptian montmorillonite (EM, a clay mineral) in the nile tilapia fish, *Oreochromis niloticus* for the adsorption of sterigmatocystin (Stg, a mycotoxinaflatoxin). They found increase in frequency of micronuclei in fishes treated with Stg, whereas no significant difference in the groups treated with EM alone and in control. They also detected that significant decrease in the number of micronuclei in case of fishes treated with EM and Stg.

Cavas and Ergene-Gozukara (2005a) evaluated the effect of petroleum refinery and chromium processing plant waste effluents for micronuclei induction and nuclear abnormalities in *Oreochromis niloticus*. They found significant increase in the frequency of micronuclei in fishes treated with both the effluents. On the other hand, they observed no significant increase in frequencies of nuclear abnormalities in fish exposed to chromium processing plant waste effluent with respect to control, whereas fishes treated with high dose of petroleum refinery effluent, showed significantly increase in nuclear abnormalities. They concluded that petroleum refinery effluent was more toxic than chromium processing plant effluent.

Bagdonas and Vosyliene (2006) subjected *Oncorhynchus mykiss* to three concentrations of copper sulphate (0.04, 0.08 and 0.16 mg/l) and found concentration dependent response for micronuclei. Matsumoto *et al.* (2006) evaluated the effect of water contaminated with tannery industrial effluents on a fish *Oreochromis niloticus* collected from three locations, one at 200m

upstream of the tannery effluents discharge site, second at tannery effluent discharge site and third at 500m downstream of the effluent discharge site of Corrego dos Bagres stream. They found more micronuclei induction and nuclear abnormalities at discharge site than downstream and upstream of the river.

Ergene-Gozukara *et al.* (2007) detected the effect of polluted Goksu delta river on three fish species, *Clarias gariepinus*, *Alburnus orontis*, and *Mugil cephalus* collected from the sites Paradeniz (PD) and Akgol Lake (AG) in Turkey. The comparative analysis of the study indicated that both micronuclei and nuclear abnormalities were significantly higher in fishes from AG lagoon than in fish from PD lagoon except frequency of nuclear abnormalities in *Clarias gariepinus*. Out of these, *Clarias gariepinus* possessed the high frequencies of micronuclei and nuclear abnormalities, while the *Alburnus orontis* had the lowest frequencies.

Cavas and Konen (2007) monitored the effect of glyphosate formulations on the peripheral blood erythrocytes on a goldfish, *Carassius auratus* and cyclophosphamide used as positive control. They observed significant increase in micronuclei frequencies in fishes treated with the glyphosate, while nuclear abnormalities like binucleated and notched nuclei were predominant as compared to lobed and blebbed nuclei. Moreover, individual nuclear abnormality did not possess any significant difference as compared to positive control, whereas total nuclear abnormalities showed dose and time dependent response.

Radwan and Ghaly (2008) measured the antimutagenic effect of neem leaves extract against sodium arsenate mutagenicity in a freshwater fish, *Clarias lazera.* They found neem extract induced non-significant increase in the micronuclei as compared to control, while significant increase in the micronuclei occurred in fishes treated with sodium arsenate. Moreover, neem extract significantly reduced the frequency of micronuclei induced by sodium arsenate. Normann *et al.* (2008) investigated the effect of potassium dichromate in armored catfish, *Hypostomus plecotomus* for the micronuclei test. They

confirmed the clastogenic effect of potassium dichromate as the frequency of micronuclei was increased in treated groups as compared to control.

Ventura *et al.* (2008) studied micronuclei and nuclear abnormalities in *Oreochromis niloticus* exposed to three concentrations (6.25, 12.5 and 25 µg/L for 72h) of atrazine herbicide. They observed significant rate of micronuclei and nuclear abnormalities for all the concentrations. Hafez (2009) evaluated the genotoxicity and cytotoxicity induced by the pollutants present in four different sites at Abu-qir bay on a fish, *Mugil cephalus* and selecting the. He found formation of micronuclei, nuclear buds, binucleate cells and fragmented apoptotic cells in the fishes and confirmed that location number 4, El-Tabia pumping station to be highly polluted.

Kirschbaum *et al.* (2009) compared the effect of pollutants on a fat snook, *Centropomus parallelus* from Sao Vicente estuary (polluted with effluents of industries, sewage outfall, stormwater drainage, legal landfills and dumping sites) and Cananeia estuary (non-polluted) of Brazil. They found high frequency of micronuclei and nuclear abnormalities in Sao Vicente estuary because of the presence of pollutants.

Yadav *et al.* (2010) assessed the effect of butachlor (1.0 ppm) for 24h, 48h, 72h and 96h in a freshwater fish, *Cirrhinus mrigala.* They found number of micronuclei initially increased at 48h followed by appearance of broken egg nuclei and then both decreased upto 96h. The effect was dose and time dependent.

Saleh (2010) evaluated the effect of polluted water of Uluabat lake on the peripheral blood erythrocytes of *Cyprinus carpio.* Fishes were collected three times per year and named as group I, group II and group III. They found one micronuclei per cell in all the groups, whereas two and three micronuclei were occurred abundantly in groups II and III. They concluded that variation in micronuclei frequency was due to home wastes and other pollutants discharged into the lake.

Malla *et al.* (2011) monitored the induction of micronuclei in a freshwater catfish, *Heteropneuste fossilis* exposed to synthetic sindoor. They

found dose dependent response for the micronuclei. Parveen and Shadab (2011) determined the effect of (1.0, 2.0 and 4.0 ppm) malathion, an insecticide for 96h on a fish, *Channa punctatus*. They found dose dependent response with respect to micronuclei.

Guner and Muranli (2011) reported induction of micronuclei and nuclear abnormalities in *Gambusia affinis* exposed to copper (0.1 ppm), cadmium (1 ppm) and Cu-Cd combination (0.1 ppm Cu + 0.1 ppm Cd) for 2 weeks. They found significant increase in micronuclei and nuclear abnormalities in the Cu-Cd combination group as compared to individual Cu and Cd treated groups.

Da-Rocha *et al.* (2011) examined the micronuclei and nuclear abnormalities in *Oreochromis niloticus* exposed to potassium dichromate (12 mg/L) and in water (negative control) for 24h and 48h. They found significant increase in micronuclei and nuclear aberrations after 48h. Bucker *et al.* (2012) studied micronuclei and nuclear abnormalities in erythrocytes of the amazonian electric fish, *Apteronotus bonapartii* exposed to benzene (10 ppm and 25 ppm) for 24h, 48h, 72h and 96h. They found micronuclei in 25 ppm concentration at 72h and 96h, while nuclear deformities were observed in 10 ppm concentration at 96h.

Walia *et al.* (2013a) reported the erythrocyte aberrations in *Labeo rohita* exposed to three sublethal concentrations (3.53%, 1.76% and 0.88 %) based on 96h LC50 value of tannery industrial effluent for 24h, 48h, 72h and 96h. They observed eleven types of erythrocyte abnormalities (5 nuclear and 6 cellular). Nuclear abnormalities included nuclear extrusion, blebbed, binucleated, lobed and notched nuclei, whereas cellular abnormalities included enucleated, vacuolate, deformed, echinocytic, spindle shaped and apoptotic cells. They concluded that 3.53% concentration to be highly toxic followed by 1.76%, while 0.88% concentration can act as a safe disposal concentration.

Kaur *et al.*, (2013c, d) examined the micronuclei induction and erythrocyte aberrations in *Cirrhinus mrigala* exposed to three sublethal concentrations (24.48%, 12.24% and 6.12%) of dyeing industrial effluent for

24h, 48h, 72h and 96h. The results showed that at higher concentration (24.48%) and exposure time (96h), there was maximum number of micronuclei in erythrocytes as compared to other concentrations at various time durations as well as in control, whereas eleven types of erythrocyte abnormalities were observed. Nuclear abnormalities included nuclear extrusion, blebbed, binucleate, lobed, notched nuclei and cellular abnormalities included enucleated, vacuolated, deformed, echinocytic, spindle shaped and apoptotic cells. Concentration 12.24% and 24.48% proved to be more toxic. In 6.12% concentration, abnormalities decreased at 96h. They concluded clastogenic effects on the erythrocytes of *Cirrhinus mrigala*.

Praveena *et al.* (2014) studied the micronuclei and erythrocytes abnormalities in a freshwater fish, *Labeo rohita* exposed to sublethal concentration 10ppm ($1/10^{th}$ of 96h LC_{50}) of potassium dichromate to group II for 7 days, group III for 30 days and group I as control. They found erythrocyte abnormalities like binucleated, notched, lobed, vacuolated nucleus, echinocyte, microcyte and enucleus in both the treated groups. They concluded dose and time dependent response with respect to control.

Aita (2014) detected the micronuclei and nuclear abnormalities induced by phenol in *Clarias gariepinus.* She divided the fishes into three groups: Group I (control), Group II phenol (7 mg/l) and Group III phenol (12 mg/l) for 2, 7 and 14 days. There was significant increase in frequencies of micronuclei in erythrocytes and gills at the higher dose, whereas nuclear abnormalities like blebbed, lobed, binucleated and notched nuclei were observed.

Srivastava and Singh (2015) examined the micronuclei and erythrocytes abnormalities in fish blood caused by agricultural fungicide (Propiconazole). Fishes were divided into four groups. Group I as negative control (water), group II as positive control (4 mg/l cyclophosphamide), Group III exposed to 1.11 mg/l Propiconazole and Group IV to 2.23 mg/l Propiconazole. There were increased frequencies of MN after 48h in Group III and Group IV. They also concluded that in the Group IV, the frequencies of MN and other nuclear

anomalies were higher than group III. Major nuclear abnormalities were lobbed nuclei, notched nuclei, nuclear buds and nucleoplasmic bridge.

Jagruti (2015) studied the effect of various concentrations of RR 120 azo dye (10, 20, 30, and 40 mg.l/1 in water) for 96h in peripheral blood samples and blood smears of gills and kidney in the fingerlings of *Catla catla*. Fingerlings exposed to different concentrations of RR 120 showed marked changes both in their blood cell and nuclear morphology as compared to control. She observed presence of micronuclei, nuclear buds, fragmented apoptotic cells and binucleated cells. She recorded time and concentration dependent response.

Walia *et al.* (2015) reported the induction of micronuclei in *Labeo rohita* exposed to three sublethal concentrations (3.53%, 1.76% and 0.88%) based on 96h LC_{50} value of tannery industrial effluent for 24h, 48h, 72h and 96h. They found predominant presence of one micronucleus as compared to two and three to five micronuclei in erythrocytes of treated fishes. They concluded that 3.53% concentration to be highly toxic followed by 1.76%, while 0.88% concentration can act as a safe disposal concentration.

Histopathology:

Histopathology has been accepted as an important tool in medical science for many years. It is also used extensively in bio-medical toxicology to determine the permissible limits of environments pollutants, drugs, food/ cosmetic additives and other chemicals. This technique is used to relate the concentration of toxicant in the water and its effect on the target organs of the fish. The impact of toxic chemicals on the histology of organs was reported for the first time by Mathur (1962) and Geoffery (1976). Later, Bernet *et al.* (1999) standardized the method to assess the extent of damage caused by the toxicants and compared the extent of damage among different organs.

Sastry and Gupta (1978a) observed significant histopathological changes in the liver of snakehead fish, *Channa punctatus* due to the effect of 0.3 mg/l mercuric chloride for 30 days. The liver showed hepatocyte granulation, vacuolization of the cytoplasm, hypertrophy of the nucleus, necrosis, fatty

infiltrations, glycogen depletion and cirrhosis. Later, Sastry and Gupta (1978b) also studied the effect of 6.8 mg/l of lead nitrate for more than 125 days on the liver of same fish and noticed liver cord disarray, necrosis, inflammation of portal area, hardening of connective tissue, shrinkage of nuclei and septa formation around the blood vessels.

Sastry and Sharma (1979) exposed *Channa punctatus* to sublethal concentration (10.01) mg/l) of endrin and observed hypertrophy of hepatic cell, liver disarray cord, vacuolization of cytoplasm, necrosis, rupture of hepatic cell membrane and necrosis of central lobular area.

Dixon and Leduc (1981) demonstrated the effect of various concentrations (0.01, 0.02, or 0.03 mg/l) of hydrogen cyanide on the liver of juvenile rainbow trout, *Salmo gairdneri* for 18 days. They detected widespread degenerative necrosis of hepatocytes in all the concentrations, while it was more intense at higher concentration but necrosis started at even low concentration.

Dietrich and Schlatter (1989) documented the histopathology of gills caused by acidity of alpine lakes in Switzerland. They selected one and two year old brown trout, *Salmo gairdneri* from Laiozza Lake contained aluminum concentration (121± 28 µg aluminium/l) with low pH (5.41± 0.21). The gills showed mucous clogging and epithelium damage due to the presence of aluminum at low pH, which was toxic to the fish.

Virk *et al.* (1987) studied the changes in liver of the fish, *Mystus tengara* treated with 0.0015 ppm endrin and 5 ppm carbaryl for 28 days. They observed compact mass of cells due to loss of polygonal structure of the hepatic cells, necrosis, atrophy, vacuolization and splitting at few regions of liver tissue.

Reimschuessel *et al.* (1989) observed the histopathological effect in kidney due to intraperitonial injection of hexachlorobutadiene (sublethal dose, 500 mg/kg) daily for one week on *Carassius auratus*. After 6h, no damage was noticed, while after 24h, cytoplasmic vacuolation and necrosis occurred in the renal tubules, which particularly localized at second (P2) and third (P3)

segments of the proximal tubules. By the sixth day, the first segment (P1) of the proximal tubule showed small cytoplasmic vacuoles with damaged lumen of the renal tubules, which persisted upto 7^{th} day.

Kohler (1990) examined the liver abnormalities in flounder fish, *Platichthys flesus* caught from different contaminated areas of Elbe estuary. Heavy steatosis in the liver of flounder with significantly enlarged hepatocytes and nuclei was seen at station 1, while moderately enlarged hepatocytes with basophilic cytoplasm, dark granules and prominent melano-macrophage centres were observed at station 2.

Narain and Singh (1991) reported histopathological lesions in the liver and kidney of *Heteropneustes fossilis* subjected to endosulfan. Fishes were exposed to the 96h LC_{50} (0.007-0.013 ppm) of the endosulfan for 4 days and liver showed extensive degeneration of cytoplasm, constriction of bile duct lumen, pyknosis of nuclei and loss of glycogen, whereas kidney possessed shrinkage of glomeruli, cytoplasmic damage, epithelial desquamation in tubules and extensive degeneration of hematopoietic stroma.

Simpson and Hutchinson (1992) examined the histopathological alterations in the dab, *Limanda limanda* along the pollution gradients in the southern North Sea. Fresh female dab, 17 to 27 cm in length were sampled along the 200 km transect (Stns 3, 5, 6, 7**,** 8 and 9) extending from the Elbe estuary region (Stn 3) to the northwestern region of the Dogger Bank (Stn 9) in the southern North Sea. These sites contained high amount of organochlorines, petroleum, hydrocarbons and metals, which were high at Stn 3 and less at Stn 7. In the liver, greater degree of hepatocellular cytoplasmic vacuolization was seen at Stn 3 and 9, while haemorrhagic foci, proliferation of melano-macrophage centres, hepatocyte necrosis and infiltration were noticed at other stations. In the kidney, the prevalence of proteinaceous/cellular debris in Bowman's space of renal glomeruli was significantly greater at Stn 3 followed by Stn 9 < Stn 5 < Stn 6. Station 7 was least affected.

Wilson and Taylor (1993) exposed adult rainbow trout, *Oncorhynchus mykiss* to acute concentration (4.9 µmol) of copper solution for 24h. They

detected internal hypoxia resulted in cell swelling, thickening/curling of lamellae and haematomas in the gills of fish.

Johnson *et al.* (1993) studied the effect of chemical contaminants on the liver of winter flounder, *Pleuronectes americanus* from the northeast coast of the United States and selected 22 sites for the experiment. Fourteen of these sites, were located in/near urban embayments, while the remaining eight sites were located in non-urban embayment, among these, two (Rocky Point, Long Island Sound and Plymouth Entrance, Massachusetts Bay) were served as reference sites. They monitored hydropic vacuolization, prevalence of necrotic lesions and neoplasm in the liver. They concluded that risk of hepatic diseases was increased with the age, whereas lesion prevalence was higher in fishes collected in the spring as compared to winter.

Khan *et al.* (1994) recorded the histopathological changes in gills and liver of winter flounder, *Pleuronectes americanus* captured from a site located 1 km away at port harmon, United States, received pulp and papermill effluents and from reference site without any contamination. Severe hyperplasia of gills, focal vacuolization in liver were more pronounced alterations in adult fishes than in juvenile fishes.

Banerjee and Bhattacharya (1994) examined histopathological changes in kidney of *Channa punctatus* induced by elsan (211 ppb), mercuric chloride (16.7 ppb) and aqueous ammonia (15.64 ppm) for 7, 28, 63 and 90 days. Kidney lesions like dilation of Bowman's capsule and decrease in the dimension of Bowman's capsule were observed in all durations of elsan and mercuric chloride, whereas the increase was intense in ammonia concentration on 28 day. They noticed renal lesions like tubular epithelial degeneration and karyolysis. They also concluded that chronic nonlethal exposure to elsan, mercuric chloride affected the both endocrine and excretory parts of the kidney, while ammonia specifically damaged the excretory part of the kidney.

Paul and Banarjee (1996) exposed gills of *Heteropneustes fossilis* to sublethal concentration (0.2 g/ l) of ammonium sulphate for 24h. They observed necrosis, shedding of epithelial cells of secondary lamellae followed

by increased haemorrhages, loss of secondary lamellae, hyperplasia and fusion of secondary lamellae in the gills.

Husoy *et al.* (1996) reported the histolopathological changes in liver of atlantic cod, *Gadus morhua* and european flounder, *Platichthys flesus* from contaminated sediments of river Sorfjorden, Norwa for three months (August to November, 1992). The innermost part of the fjord (stations l-3) is contaminated by smelter industries in Odda and Tyssedal, while Station 4 (Lofthus) was used as a reference station. They observed increased lipid/glycogen multifocal distribution of vacuolated hepatocytes and focal congestion of sinusoids in the liver of flounder from stations 1-3, while increased density of melano-macrophage centers was seen in flounder at station 1. They concluded that station 1 was more contaminated.

Huang *et al.* (1997) evaluated the histopathology of liver in the spotted gar fish, *Lepisosteus oculatus* from the lower Mississippi River basin. The selected the site Devil's Swamp (DS) situated on the east bank of Mississippi River just northwest of Baton Rouge, Louisiana, which contained chlorinated hydrocarbons, (hexachlorobenzene, HCB and hexachlorobutadiene, HCBD) and variety of heavy metals and other was pristine control site, Tunica Swamp (TS) approximately, 30 miles upstream from DS near St. Francisville, Louisiana. Fishes were caught from both the sites and abundance of melanin-rich macrophage centers (MMC) in the liver was seen.

Singhal and Jain (1997) examined the histopathological changes in kidney of *Cyprinus carpio* exposed to (58µg/l, 43.5 µg/l, 29.0 µg/l and 14.5 µg/l concentrations) of cadmium chloride for acute (7-14 days) and chronic (2 and 4 weeks) toxicity. In 29 µg/l concentration, after 7 days, they observed pyknosis of nucleus, necrosis and reduced cytoplasm of lymphoidal cells in walls of nephrons, while after 14 days, broken and swollen uriniferous tubules were seen. Chronic exposure of four weeks, at 43.5 µg/l concentration caused degeneration of tubules, thick glomerular basement membrane, reduction in the number of collecting duct, extreme swelling, disintegration of the renal tubules, displacement of nucleus in the renal tubules and narrowing of lumen of

Bowman's capsule were recorded. At 58µg/l concentration, after two weeks, coagulative necrosis with cell nuclei along with pyknosis, karyohexis and fragmentation of nuclear membrane were noticed, whereas after 4 weeks, extreme necrosis and inflammation of tissue were seen.

Teh *et al.* (1997) detected the changes in liver and gills of red breast sunfish, *Lepomis auritus* exposed to different types of contaminants. They selected four sites, first site was stream called east fork popular creek (EFPC) contained discharge of nuclear weapon, second site was pigeon river (PR) received bleached kraft mill effluent (BKME), third site was Hartwell reservoir (HR) contained high level of PCBs, while Brushy fork creek (BFC), little river (LR) and Tugaloo river (TR) were served as reference sites. They observed variable glycogen depletion, fat vacuolization, occasional hepatocellular necrosis, macrophage aggregation, clubbing, fusion of lamellae and hyperplasia in the fishes of all contaminated sites, whereas fatty degeneration of hepatocytes, vacuolated cells and necrosis were seen in the liver of PR stream and HR reservoir. Few histopatological alterations were also noticed in the fishes of reference sites, however, these changes were less severe than contaminated sites.

Schwaiger *et al*. (1997) examined the changes in juvenile brown trout, *Salmo trutta* and adult loach, *Barbatula barnatula* collected from site A (Krahenbach) and site K (Korsch) after 6 weeks and 21 weeks of each season. Both the fishes of Korsch water stream in warm season showed granulomatous nephritis, degenerative and necrotic changes in the kidney, while reduction in the glycogen of hepatocytes, multifocal inflammatory process in liver and focal proliferations of primary and secondary lamellae epithelial cells, fusion of secondary lamellae, mucous cell hyperplasia, hydropic degeneration of respiratory epithelial cells in the gills. In Krahenbach stream, both the fishes did not show any seasonal variation but they possessed primarily vacuolization, hypertrophy, necrosis, dilation of tubules and accumulation of protinaceous material in tubules of kidney, while glycogen depletion and inflammatory fibrotic changes in the liver and focal hyperplasia of epithelial and mucous

cells in the gills. They concluded that all the tissues were more damaged in the Korsch River than Krahenbach river stream.

Frances *et al.* (1998) documented the effect of overdose (50 mg/l) of benzocaine (ethyl *p*- aminobenzoate) on silver perch, *Bidyanus bidyanus* and observed lamellar fusion, aneurysm and hyperplasia in the gills. Marlasca and Sampera (1998) studied the histopathological changes in rainbow trout, *Oncorhynchus mykiss* exposed to 0.3 % textile industry effluent based on 96h LC_{50} value for 15, 30, 45 and 60 days. They noticed abnormal level of lipids, nucleus anaplasia, loss of hepatic arrangement and two cords like arrangements in all the durations, while after 45 days, hepatic cells showed sinusoids inflammation.

Escher *et al.* (1999) assessed the effect of treated wastewater from a sewage plant on gills, kidney and liver of brown trout, *Salmo trutta*. Two cages were placed in the river RWW (river water plus wastewater), RWO (river water only) in the river "Alte Aare", 20m upstream of the effluent from the sewage plant of Lyss and TWR (tap water) as control for 56, 62 and 66 days. Gills of RWW showed distortion of lamellae, fusion of the secondary lamellae, hypertrophy, oedema, hyperplasia, whereas few pyknotic nuclei were present in RWO fish. Livers of both RWO and RWW sites possessed mild hyperplasia, infiltration of lymphocytes and dilatation of liver sinusoids, while necrosis, cytoplasmatic vacuolization, karyolysis and karyorrhexis were seen only in RWW. Kidney of both RWO and RWW sites revealed necrosis and accumulations of melano-macrophages, whereas mild interstitial fibrosis and proliferation of hematopoietic tissue, hyaline droplet degeneration and increased number of pyknotic nuclei were observed at RWO site.

Couillard *et al.* (1999) evaluated the histopathological changes in atlantic tomocod, *Microgandus tomcod* collected from Restigouche estuary, Miramichi estuary and Pictou harbor received pulp and paper mill effluent, while Kouchibouguac and Margaree estuaries were as reference site. Fishes showed localized granulomatous inflammation, macrophage aggregates and

focal granulomas. These alterations were more at Miramichi estuary as compared to other sities.

Das and Mukherjee (2000) studied the histopathological effect on gills, kidney and liver of *Labeo rohita* exposed to 0.35ppm and 1.73ppm of hexachlorocyclohexane for 45 days. In 0.35 ppm concentration, mild congestion of blood cells in liver, while mild degenerative changes of tubular epithelium in kidney and mild congestion of blood cell in primary lamellae of gills were noticed. At 1.73 ppm concentration, marked swelling of the hepatocytes in places of diffuse necrosis, distended sinusoids and central veins severely damaged in the liver were recorded. Karyorrhexis, karyolysis and dilated lumens of tubules with infiltration of mononuclear cells in the kidney and hyperplasia with fusion of primary lamellae in gills were observed.

Bhuiyan *et al.* (2001) detected the effect of sumithion (100ppm) on the liver and kidney of the spotted murrel, *Channa punctatus* for 7 days. They observed rupture of blood vessel, haemorrhages, pyknosis, mild necrosis and vacuolization in the liver, while vacuolization, necrosis, pyknosis, haemorrhages and degeneration of tubules and hematopoietic cells were seen in the kidney.

Rahman *et al.* (2002) examined the histopathological effect of diazinon 60 EC on the liver and kidney of *Anabas testudineus, Channa punctatus* and *Barbodes gonionotus. Anabas testudineus* (1.13 and 3.75 ppm), *Channa punctatus* and *Barbodes gonionotus* (1.13 and 2.26 ppm) were exposed to sublethal concentrations based on their 96h LC_{50} values for 7 days. In all the fishes, at 1.13 ppm concentration, hypertrophy of hepatocytes, necrosis and vacuolization, while at 3.75 ppm and 2.26 ppm concentrations, pyknosis, necrotic, hypertrophy, haemorrhage and vacuolization were seen in liver. In kidney, at 1.13 ppm concentration, all the fishes possessed necrosis, degenerated tubules, pyknosis and haemorrhages except in *Barbodes gonionotus,* which included melanogenesis with inflammatory cells. At 3.37 ppm concentration, kidney of *Anabas testudineus* showed degeneration of kidney tubules, necrosis, pyknosis and haemorrhages, while at 2.26 ppm

concentration, *Channa punctatus* and *Barbodes gonionotus* showed severe necrosis, vacuolization and tubular degeneration.

Hossain *et al.* (2002) studied the effect of dimecron 100 SCW (0.5 and 5.0 ppm) on *Anabas testudineus*, *Channa punctatus* and *Barbodes gonionotus* for 7 days. In 0.5 ppm, liver of *Anabas testudineus* caused hypertrophy, haemorrhages, pyknosis and necrosis, while in *Channa punctatus* mild necrosis and vacuolization were occurred and in *Barbodes gonionotus* hypertrophy, melanogenesis, mild pyknosis and necrosis were observed. Kidney in *Anabas testudineus* showed mild pyknosis, mild degeneration, while in *Channa punctatus* necrosis, vacuolization, tubular degeneration were observed and in *Barbodes gonionotus* mild pyknosis and necrosis were recorded. Fishes exposed to 5.0 ppm, liver of *Anabas testudineus* caused vacuolization, severe necrosis, pyknosis and haemorrhages, while in *Channa punctatus* melanogenesis, pyknosis, severe necrosis, release of blood cells, rupture of blood vessel were seen and in *Barbodes gonionotus* hypertrophy, melanogenesis, mild pyknosis, necrosis and vacuolization were noticed. Kidney in *Anabas testudineus* showed necrosis, pyknosis, haemorrhages, vacuolization and tubular degeneration, while in *Channa punctatus* mild pyknosis, vacuolization, tubular/hematopoietic tissue degeneration, rupture of blood vessel, haemorrhages were noted and in *Barbodes gonionotus* necrosis, haemorrhages, severe tubular, vacuolization and hematopoietic tissue degeneration were recorded.

Thophon *et al.* (2003) reported the histopathological alterations in gills, kidney and liver of white seabass, *Lates calcarifer* on acute exposure (10 mg/l for 96h) and subchronic exposure (0.8 mg/l for 3 months) to cadmium. After 96h, gills showed oedema, aneurysm, hypertrophy, hyperplasia and blood congestion, while in liver, blood congestion, accumulation of dark granules, vacuolization and hydrophic swelling of hepatocytes were seen and in kidney, hydropic swelling, hyaline droplets, hypertrophy, pyknosis, accumulation of dark granules were present. In subchronic exposure, oedema, hypertrophy and hyperplasia were noticed in gills, while hydrophic swelling of hepatocytes,

vacuolization and dark granules in hepatocytes were observed in liver and hypertrophy, pyknotic nuclei, hyaline droplet in tubular cells were recorded in kidney.

Kadry *et al.* (2003) evaluated the damage in the kidney of Mugil fish, *Liza Ramada* from polluted Lake Manzalah in Egypt from autumn to summer. They observed mild increase in Bowman's capsule space, degenerated hematopoietic tissue, widened lumens of tubules, degenerated epithelial cells with vacuolated cytoplasm and pyknotic nuclei in autumn, while necrosis of hematopoietic tissue, distortion of glomerular capillaries, mesangiolysis, fibrocytes, inflammatory cells, shrinkage and degeneration of Bowman's capsule and renal tubules in winter. They also recorded loss of normal architecture of renal tubules, vacuolated cytoplasm, haemolysis, pyknosis, shrunken glomeruli, hypercellular glomeruli, atrophied nuclei and necrotic tubules and hematopoietic tissue in spring, whereas partially or completely distorted corpuscles, obliteration of Bowman's capsule, vacuolization in glomerulus, renal tubules degeneration, necrosis and complete lysis of renal tubules in summer.

Ortiz *et al*. (2003) examined the histopathological changes induced by accidental discharge of lindane (γ-HCH) into the Barbate river (spain) on gills, liver and kidney of *Mugil* species, *Cyprinus carpio* and *Barbus* species. The main histopathological alterations were same in all the fishes. They observed dilation of blood capillaries, hyperplasia of secondary lamellae, necrosis, shortening of secondary lamellae, abnormal swelling of epithelium, fusion of secondary lamellae and excessive mucus secretion in gills, while steatosis, increased basophilia within cytoplasm of hepatocytes, hepatocellular necrosis with parenchyma vacuolization, hypertrophy of hepatocytes, haemorrhages and widening of blood sinusoids were seen in liver and disintegration of convoluted tubules, intracytoplasmic vacuoles, shrinkage of glomerulus and increased space within the Bowman's capsule were detected in kidney.

Khan *et al.* (2004) reported histopathological effects on kidney and gills of grass carp, *Ctenopharyngodon idella* on acute and chronic exposure of

mercuric chloride. For acute exposure, fishes were divided into four groups A (Control), B (0.4 mg/l), C (0.5 mg/l) and D (0.6 mg/l) for 96h and for chronic exposure, E (Control), F, G, H with sublethal dose of 0.3 mg/l for 4, 8, 12 and 16 days, respectively. In acute phase, no histopathological lesions were noticed in gills and kidney after 48h in group B, while in group C and D, gills showed moderate hypertrophy, epithelial degeneration and necrosis and no change was observed in kidney tissue. After 96h, in kidney of B, C, D groups, pyknosis, necrosis, epithelial degeneration, intracellular widening were present, while hypertrophy of lamellae, epithelial degeneration, epithelial necrosis and club shaped lamellae were seen in gills. They concluded that changes in both the fishes were mild (B), moderate (C) and severe (D) in nature. In chronic exposure, groups F, G, H showed thickening of renal tubules, nuclear pyknosis, degeneration and disorganization of hematopoietic tissue in kidney, whereas hyperplasia of gill epithelium, fusion of secondary lamellae, destruction of respiratory epithelium and formation of club shaped lamellae were detected in gills. They also concluded mild (F), moderate (G) and severe (H) in nature.

Adeyemo (2005) evaluated the histopathological changes in liver of fish *Clarias gariepinus* exposed to Cassava mill effluent for 96h. The fishes were divided into 5 groups A (2 ml), B (5 ml), C (10 ml), D (15 ml) and E (Control). No marked histopathological lesions were seen in the fishes subjected to low concentrations (2 and 5 ml), whereas in higher concentrations (10 ml and 15 ml) necrosis, hypertrophy and vacuolization of hepatocytes were recorded.

Gupta and Kumar (2006) detected the histopathological lesions in gills and kidney of *Cirrhinus mrigala* exposed to sublethal concentration (240 µg/l) of mercuric sulphate for 96h. Treated fishes showed fusion of secondary lamellae at distal region, while occlusion of renal tubules, glomeruli with blood cells, necrosis of renal tubules and decreased hematopoietic tissue were noticed in kidney.

Gupta and Shrivastva (2006) examined the effect of three sublethal concentrations (Group II-10mg/l, Group III- 15mg/l and Group IV- 25mg/l) of zinc on kidney tissue of *Channa punctatus* for 8, 10 and 15 days.

Histopathological changes were appeared after 8 days and continued till 15 days. These changes included renal tubules expansion, lose cellular integrity, oedema and hypertrophied nuclei, while glomerulus showed vacuolization, necrosis, pyknotic nuclei and disorganized blood capillaries. They concluded that tissue damage showed concentration and time dependent response.

Athikesavan *et al.* (2006) studied the histopathological changes in gills, liver and kidney of freshwater fish, *Hypophthalmichthys molitrix* treated with sublethal concentrations of nickel (5.7 mg/l) for 10, 20 and 30 days. After 10 days, they observed infused secondary lamellae in gills, while degeneration of blood vessels, vacuolization, hypertrophy in liver and hyperplasia, hypertrophic nuclei and haemolysis in kidney. After 20 days, pronounced changes like distortion of secondary lamellae, fusion of secondary lamellae, hypertrophy, hyperplasia in gills, while swollen hepatocytes, acute inflammation, necrosis, vacuolization and degeneration of blood vessels in liver and cytoplasmolysis, karyolysis and vacuolization in kidney were noticed. After 30 days, severe erosion, degeneration of epithelium and aggregation of blood corpuscles in gills, while lose cytoplasmic density with accumulation of pyknotic nuclei, degeneration of vacuoles and necrosis in liver and syncytical cells, pyknotic nuclei, disrupted glomerulus and enlarged uriniferous tubules in kidney were seen.

Silva and Martinez (2007) examined the histopathology in kidney of a neotropical fish, *Astyanax altiparanae* from an disturbed urban stream and clean site as control. They selected three sites, site A, 3.7km from stream source and received effluent from coffee factory and agro-industrial wastes, site B, 1.1 km downstream of site A and received domestic sewage and agrochemicals wastes and site C, 2.7km downstream of site B receiving effluent from car battery factory. They observed minor morphological changes in kidney of reference site, while severe histopathological alterations like cytoplasmic vacuolization, hyaine droplet degeneration, hypertrophy, haemorrhages, dilation of capillaries, reduction in Bowman's space, necrosis and occlusion of renal tubules in fishes were recorded from A, B, C sites.

Joshi *et al.* (2007) detected histopathological changes in liver of fish *Heteropneustes fossilis* exposed to 0.012ppm concentration of cypermethrin for 20, 30, 40 and 60 days. After 20 days, hepatocytes loose their polygonal shape, cloudy swelling, vacuolization and pyknosis were present, while after 30 days, pronounced changes in hepatocytes like necrosis, pyknosis were seen. However, after 40 days, intense vacuolization in cytoplasm and pynotic nuclei were noticed, whereas after 60 days, extensive vacuolization, pyknosis and necrosis were evident. They concluded that histopathological changes were more severe with increase in exposure period.

Figueiredo-Fernandes *et al.* (2007) evaluated histopathological changes in liver and gills of *Oreochromis niloticus* exposed to sublethal concentrations (0.5, 1.0 and 2.5 mg/l) of copper sulphate for 21 days. In all the concentrations, gills showed lifting of the lamellar epithelium, oedema, lamellar fusion, aneurism, epithelium proliferation and lamellar vasodilatation, while hepatic parenchyma, lower eosinophilia, hepatocellular necrosis and an increase of cytoplasmic vacuolization were recorded in liver. They illustrated that gills produced evident damage at higher concentration (2.5mg/l), whereas it was pronounced at 1.0 mg/l and 2.5 mg/l in liver.

Velmurugan *et al.* (2007) studied the effect of sublethal concentrations of fenvalerate (Group I -1.5 ppb and Group II -3.00 ppb) on gills, liver and kidney tissues of freshwater fish, *Cirrhinus mrigala*. Group III was set as control. Gills showed epithelial hyperplasia, desquamation, necrosis and lamellar fusions at low concentrations, while desquamation, swelling at the tips of secondary lamellae, necrosis, curling of secondary lamellae, oedema, severe lamellar fusion and hyperplasia were seen at high concentration. In kidney, contraction of the glomerulus, increased Bowman's capsule space, pyknotic nuclei in hematopoietic tissue were observed at low concentration, whereas narrowing of tubular lumen, hypertrophied epithelial cells of renal tubules, necrosis of tubular epithelium, pyknotic nuclei in hematopoietic tissue were noticed at high concentration. Liver included congestion, cloudy swelling of

hepatocytes, at low concentration, while congestion, cloudy swelling of hepatocytes and focal necrosis at high concentration.

Moneim *et al.* (2008) determined the histopathological effects in catfish, *Clarias lazera* exposed to dyestuff and chemical wastewater (3.12 %, 6.25 % and 12.5%) for 28 days. In 3.12% concentration, haemorrhages of pillar cells, dilation of blood capillaries, aneurism, fusion of lamellae, hypertrophy, necrosis in secondary lamellae and rupture of epithelium and in 6.25%, lamellar fusion, edema with rupture pillar cells, hypertrophy and hyperplasia of epithelial cells, while in 12.5% concentration, necrosis of pillar cells, extensive dilation of capillary wall, aneurism and hyperplasia in gills were seen. In liver, 3.12%, severe disorganization of hepatic cords, damaged cell membrane, extensive necrosis of hepatocytes and cytoplasmic vacuolization and in 6.25%, swelling of hepatocytes, congestion of sinusoids, necrosis, leucocytes infiltrations, pyknosis and hypertrophied kupffer cells, while at 12.5%, cloudy swelling, necrosis, infiltrations were observed. In kidney, at 3.12%, extensive glomeruli damage, occlusion of tubules, shrunken glomeruli, increased Bowman's capsule space, necrosis, karyolysis of nuclei and extensive fibrosis and in 6.25%, shrunken glomeruli, enlarged Bowman's space, destroyed tubular epithelium and hyaline droplet degeneration, while in 12.5%, damaged renal corpuscles with breakdown of glomeruli blood capillaries, tubular shrinkage, synclinal tubules, necrotic cells and complete lysis of cytoplasm were noticed. They depicted that these changes were increased with increase in concentration.

Bhattacharya *et al.* (2008) evaluated the histopathological alterations in liver, gills and kidney of rosy barb, *Puntius conchonius* subjected to sublethal concentrations of CCL_4 (5, 7.5 and 10 mg/l). Liver of fishes treated with concentration 5 and 7.5 mg/l showed histological lesions like focal necrosis, mononuclear lymphocyte infiltration in sinusoids, which were more severe in 7.5 mg/l, whereas in 10 mg/l, severe cell infiltrations, haemorrhages, lost hepatocytes boundaries, nuclear pyknosis, necrosis and fatty acid degeneration were recorded. In 5 mg/l, kidney revealed connective tissue with increased

mass of cell infiltrations, shrunken glomeruli and dilated Bowman's capsule, while in 7.5 and 10 mg/l, concentrations, increased masses of inflammatory cell infiltration, glomeruli and Bowman's capsules were severely degenerated. In all the concentrations, synctium was seen in the gills.

Kurtovic *et al.* (2008) compared the histopathological changes in kidney of farmed and wild European sea bass, *Dicentrarchus labrax*. The control groups were wild fishes, caught from Tarska Bay, Adriatic Sea. The kidney of farmed and wild sea bass showed atrophy of glomerulus, lysis of the glomerular tuft, inflammatory changes in tubules, necrotic tubules and melano-macrophages centers. They concluded that damage were more significant in wild fishes than farmed fishes.

El-Naggar *et al.* (2009) detected the histopathological alterations in liver of *Oreochromis niloticus* from nine different localities (El-Oqsur, El-Menia, El-Hawamdia, Shoubra El-Khema, El-Rahawy drain, Kom Hamada, Talkha, El-Serw and Faraskour) along the river Nile, Egypt. They observed cytoplasmic vacuolization, fatty degeneration, lymphocytic infiltration, hemolysis, hepatocellular focal necrosis, edema, haemorrhages and congestion in the liver of all the sites. The damage was severe in fishes of El-Hawamdia and Shoubra El-Khema sites received industrial wastes and El-Menia and El-Rahawy sites received sewage wastes.

Butchiram *et al*. (2009) studied the histopathological changes in gills, liver and kidney tissue of *Channa punctatus* exposed to alachlor (1.21mg/l) and lasso (50% EC 1.54 mg/l) for 10 days. In gills, bulging of tip of primary gill filaments, distorted secondary filament, fusion of secondary filament, pillar cell necrosis, vacuolization in secondary gill epithelium, whereas in liver degeneration of cytoplasm in hepatocytes, atrophy, formation of vacuoles, rupture in blood vessels, necrosis, disappearance of hepatocyte wall and disposition of hepatic cords and in kidney, degenerative changes in hematopoietic tissue, necrosis, cloudy swelling in renal tubules, cellular hypertrophy were recorded.

Saenphet *et al*. (2009) evaluated the histopathological alterations in gills, liver and kidney in *Anabas testudineus* (Bloch) from an unused lignite mine, Li district, Lamphun province, Thailand. They observed desquamation in secondary lamellae, lifting of the lamellar epithelium and telangiectasia in gills, while hemorrhage, blood congestion, necrotic cells and mononuclear cell focal infiltrations in liver and degeneration of epithelial cells of renal tubules, degeneration of glomeruli, hypertrophy in renal tubules, narrowing of tubular lumen and shrunken glomerulus in kidney.

Velmurugan *et al.* (2009) studied the histopathologial changes in the gills and liver tissues of freshwater fish, *Cirrhinus mrigala* exposed to sublethal concentrations (0.91 and 1.82 ppm) of dichlorvos for 10 days. In both concentrations, gills showed hyperplasia, desquamation, necrosis of epithelial, odema, lamellar fusion, collapsed secondary lamellae and curling of secondary lamellae, whereas liver included cloudy swelling of hepatocytes, congestion, vacuolar degeneration, karyolysis, karyohexis, dilation of sinusoids and nuclear hypertrophy alterations. They depicted that lesions were more severe in both tissues at high concentration than low concentration.

Al-Mansoori *et al.* (2010) detected the histopathological effect on kidney of *Carassius carassius* (juveniles) exposed to cadmium (0.2 and 0.5 ppm) and lead (15 and 30 ppm) concentrations for 8 days. Kidney showed karyohexsis, degenerating tubules and karyloysis at low concentration, while focal degeneration of tubular cells, atrophy of tubules, extensive necrosis of whole nephron, leucocyte and erythrocyte condensation at high concentration of cadmium, However, kidney included atrophy of grabuloma, karyohexis, cell cytoplasm depression in lumina at low concentration, whereas karyolysis, karyohexsis, cell degeneration, necrosis, edema and atrophy of tubules at high concentration. They concluded that lead caused more damage to kidney than cadmium.

Velma and Tchounwou (2010) determined histopathological effects on liver and kidney of goldfish, *Carassius auratus* exposed to chromium (VI). Fishes were selected as Control (0 mg/l), $LC_{12.5}$ (21.4 mg/l), LC_{25} (42.85 mg/l)

and LC_{50} (85.7 mg/l) for 96h. In control, liver showed sinusoids scattered randomly all over hepatocytes and hepatocytes arranged uniformly along its central vein. In all the concentrations, hepatocytes disruption, hepatocellular vacuolization were seen in liver, while pycknotic and partial disruption of central vein at 42.85 mg/l concentration and degeneration of liver (atrophy), central vein injuries at 85.7 mg/l concentration were noticed. In kidney, at 21.4 mg/l concentration, tubular dilation, renal tubular separation, at concentration 42.85, tubular necrosis, disintegration of tubules, degeneration of hematopoietic tissue, while at 85.7 mg/l concentration, dilation of tubules, tubular necrosis, renal tubular separation, degeneration of hematopoietic tissue were observed.

Pathan *et al.* (2010) studied the histopathological changes in the gills of freshwater fish, *Rasbora daniconius* exposed lethal concentration 9.5% (LC_{50} of 96h) for 96h and sublethal concentrations [1.9% (1/5) and 0.95% (1/10) LC_{50} of 96h] of paper mill effluent for 30 days. In 9.5% concentration, gills showed fusion and curling of secondary lamellae, reduced secondary lamellae, haemorrhages, bulging at the tip of primary lamellae, cloudy swelling in cells, ruptures in secondary lamellae and hypertrophy. However at 1.9% concentration, curling of secondary lamellae, rupture in the secondary lamellae, cloudy swelling with pyknotic nuclei, hypertrophy and widening of inter lamellae distance were recorded, while at 0.95% concentration, fusion of secondary lamellae, haemorrhages at primary lamellae, cloudy swelling with bulging at the tip of primary filament, curling of secondary lamellae, hypertrophy were seen.

Ravanaiah and Murthy (2010) detected the impact of aquaculture and industrial pollutants of Nellore district on the kidney of fish, *Tilapia mossambica*. They recoded severe histopathological changes like vacuolization of collecting tubules, shrunken and ruptured glomeruli, disintegration of hematopoietic tissue, enlarged lumen, fibrosis and aggregation of melano-macrophage centers in proximal and distal convoluted tubules.

Besirovic *et al.* (2010) evaluated the histopathological effects on kidney and gills of brown trout, *Salmo trutta* exposed to chronic exposure of cadmium (4.4 mg/kg) and zinc (11 mg/kg) for 46 week. Gills showed fusion of lamellae, while kidney included degenerative changes in the epithelial cells, vacuolization and accumulation of proteinaceous content in Bowman's capsule exposed to cadmium. On the other hand, kidney induced hyperplasia of the epithelial cells and appearance of small granules in their cytoplasm, whereas no effect was seen in gills under the influence of zinc.

Patel and Bahadur (2011) reported the histopathological alterations in liver of *Catla catla* exposed to sublethal concentrations (0.1 mg/l and 0.3 mg/l) of copper sulphate for 7, 14 days and three weeks. After 7 days, in 0.1 mg/l, cytolysis, vacuolization in perinuclear space and pyknotic nuclei, while in 0.3 mg/l, blebbing of cytoplasm, and hemorrhage in central vein were observed. After 14 days, 0.1 mg/l, dilation of sinusoids and fibrosis within sinusoids and at 0.3 mg/l, dilated sinusoids and focal necrosis were seen. However, after 3 weeks, in 0.1 mg/l, haemorrhages within sinusoids and 0.3 mg/l, remarkable increase of above mentioned alterations were recorded. They concluded time and dose dependent effect of copper sulphate.

Bhatkar (2011) studied the histopathological alterations in the liver of Indian common carp, *Labeo rohita* induced by chromium chloride in group I (6 mg/l), nickel chloride in group II (4 mg/l) and zinc chloride in group III (2 mg/l) for 10 and 30 days. After 10 days, liver of fishes of group I showed cytoplasmic damage and vacuolization, group II possessed vacuolization, appearance of tubular bodies, in filtered fats and hypertrophy, whereas in group III swelling of hepatic nuclei, disorganization of hepatic cells with edema were seen. After 30 days, in group I enlarge nuclei, elongation of blood vessels, necrosis, condensation of cytoplasm and disarray of hepatic cords, in group II degeneration of sinusoids, bleeding in the intercellular gaps and degeneration of hepatic cells, while in group III, necrosis, degeneration of sinusoids and disintegration of hepatocytes were observed.

Joshi (2011) assessed the effects of 3 mg/l of zinc sulphate for 10, 20, 30 days on liver and kidney of freshwater fish, *Clarias batrachus.* After 10 days, liver showed swelling of hyperchromatic nuclei, disorganization of hepatocytes and disarray of hepatic cord, while kidney possessed destroyed proximal tubules, disintegrated cytoplasm and interstitial tissue. After 20 days, blood coagulation, degenerated and vacuolated hepatocytes in liver, whereas haemorrhages, hypertrophied nuclei and vacuolated cells in kidney were seen. After 30 days, disintegrated cytoplasm of hepatocytes, spongy mass and acute haemorrhages in liver, while disintegration of uriniferous tubules, clumping of nuclei of epithelial cells, damage renal tubules and necrosis in kidney were recorded. He concluded that effect was more in kidney and changes showed time dependent response.

Singh (2012) detected the histopathological alterations in the kidney of *Cyprinus carpio* exposed to 0.96 mg/l dimethoate for 24h, 48h and 96h. Kidney of control was composed of numerous renal corpuscles with well defined glomeruli and renal tubules, whereas after 24h, treated fishes showed no changes, after 48h, shrinkage in glomerulus, increase Bowman's space, widen lumen and pyknosis were seen and after 96h, widen tubular lumen along with degenerative tubular epithelium, hyaline degeneration, swelling, vacuolization, shrunken glomerulus and necrosis were recorded.

Sounderraj *et al.* (2012) studied the lethal (35 ppm for 15 days) and sublethal (3.5 ppm for 30 days) effect of tannery industrial effluent on gills, kidney, liver of common carp, *Tilapia mossambica.*. At 3.5 ppm, gills showed fusion of secondary lamellae, necrosis of epithelial layer, erosion of respiratory epithelium, hypertrophy, hyperplasia and clubbing of lamellae at the tips, while in liver, damaged hepatocytes, binucleated cells and aggregation of hepatocytes were seen and in kidney, vacuolization, tubular necrosis, hypertrophy, hyperplasia, pyknosis were noticed. At 35ppm effluent, gills possessed hyper plastic cells, clubbing of lamellae, hypertrophy and erosion of respiratory epithelium, whereas in liver, vacuolization of hepatocytes, hypertrophy with

damaged hepatocytes were present and in kidney, vacuolization, hypertrophy, cytoplasmolysis and hyperplasia were observed.

Navaraj and Yasmin (2012) determined the effect of tannery industry waste (0.8%, 1, 1.2 and 1.4% based on 96h LC_{50} value) for 60 days on gills, liver and kidney of the fish, *Oreochromis mossambicus*. They observed hyperplasia, epithelial lifting, cells swellings, congestions, bending of secondary lamellae, oedema, degenerated epithelium and infiltration of leucocytes in gills, while vacuolization, fatty infiltrations, congested central vein, pyknotic nuclei and necrosis in liver and decrease of hematopoietic tissue, necrosis, enlarge sinusoids and occlusion of tubules in kidney.

Jalaludeen *et al*. (2012) studied the histopathology of the gills, liver and kidney tissues of the freshwater fish, *Tilpia mossambica* exposed to 0.084 mg/l of cadmium sulphate for 20 days. After 10^{th} day, lesion in epithelial layer, hypertrophy of mucous cells and vacuolization in gills, while proliferation in hepatic cords in liver and mild oedema, damaged renal tubule, degeneration of glomerulus in kidney were observed. After 20 days, oedema, hypertrophy, decreased number of cell, congestion, haemorrhages vacuolization, destroyed and curled secondary lamellae in gills, while marked proliferation, scattered cells with severe vacuolization in liver and severe damage and disorganization of tubules along with glomerular edema and necrosis in kidney were recorded.

Authman *et al.* (2013) demonstrated the effect of heavy metal pollution on gills and liver of nile catfish *Clarias gariepinus* collected from Delta Barrage in front of El- Kanater El- Khayria city (reference site) and El-Rahawy Drain from village in Egypt. They observed dilation, intracellular vacuolization, sloughed gill filaments, congestion in blood vessel of primary gill filament, hyperplasia, fusion of lamellae in gills, while loss of cellular architecture, vacuolar degeneration, pyknotic nuclei, focal areas of necrosis, leukocyte infiltrations, dilation of central vein, blood congestion and hyaline degeneration in the hepatic tissue of liver.

Praveena and Rao (2013) observed the histopathological alterations in kidney tissue of an Indian common carp, *Labeo rohita* due to potassium

dichromate 10ppm for 7 and 30 days. After 7 days, kidney showed necrosis in glomerulus, moderate necrosis in Bowman's capsule and necrosis in collecting tubules, while after 30 days, severe necrosis in Bowman's capsules, degenerative changes in glomerulus and necrosis in hematopoietic tissue were seen.

Al-Balawi *et al*. (2013) examined the histopathology of gills, liver and kidney of *Clarias Gariepinus* exposed to sublethal concentrations of lead acetate 5% (Group TI- 6.1 mg/l), 10 % (Group TII- 12.2 mg/l) and 20% (Group- TIII 24.4 mg/l) of the 96h LC_{50} for six weeks. Gills of group (TI-TIII) showed blood congestion, epithelial hypertrophy, epithelial lifting, vacuolar degeneration of the cytoplasm and degeneration of secondary lamellae. In (TI) hyaline degeneration, (TII and TIII) necrosis of hepatocytes nucleus and (TIII) hepatocytes with double nuclei were noticed, whereas in kidney glomerular expansion, reduction in Bowman's space in (TI and TII), tubular necrosis (TII and TIII) and blood congestion (TIII) were seen.

Rathnamma and Nagaraju (2014) evaluated the histopathological changes in liver and gills of a freshwater fish, *Cirrhinus mrigala* exposed to 1.64 mg/l sublethal concentration of chlorantraniliprole for 15 days. They recoded epithelial lifting, aneurysm, necrosis, development of vacuoles in the epithelium, hyperplasia of gill filaments, fusion of gill filaments, degeneration of secondary lamellae and pillar cells in gills, while degeneration of hepatocytes, severe necrosis and oedema in liver.

David and Kartheek (2014) studied the effect of sublethal concentration 0.2 mg/l based on 96h LC_{50} value of sodium cyanide in kidney of freshwater fish, *Cyprinus carpio* for 10 and 20 days. They observed cytoplasmic vacuolization, shrinkage in lumen, glomerular degeneration after 10 days, while inflammation, increase cytoplasmic vacuolization, blood coagulation and necrosis after 20 days.

Varadarajan *et al.* (2014) detected the sublethal effects on gills of tropical teleost fish, *Oreochromis mossambicus* exposed to phenol 3.12 mg/l and m-cresol 2.2 mg/l based on 1/10 of 96h LC_{50} for 21 days. In 3.12 mg/l,

gills showed architectural loss, necrosis, desquamation of epithelial layer, hyperplasia and telangiectasia, while in 2.2 mg/l, lamellar necrosis, lamellar shortening, telangiectasia and lamellar clubbing were recorded.

Jagruti (2015) evaluated the histopathological changes in gills and kidney in fingerlings of *Catla catla* exposed to RR 120 azo dye (10, 20, 30, and 40 mg/l in water) for 96h. They observed aneurism, hyperplasia, degenerated central axis, lifting of gill epithelium, curved secondary gill lamellae in gills, while shrunken glomeruli, increased periglomerular space, degenerated renal tubules in kidney. They concluded that histopathological changes showed time and concentration dependent response.

Sivakumar *et al.* (2015) reported various histopathological changes in gills and liver of *Danio rerio* exposed to $1/4^{th}$ (4.8 ppm) and $1/10^{th}$ (12.1 ppm) of tannery industry effluent for 90 days. They observed in 4.8ppm, mild degeneration of secondary lamellae and fusion of secondary lamellae in gills, while hepatocytes lost their polyhedral shape, cell lysis, disconnection between hepatocytes, degeneration and aggregation of the hepatocytes cells in liver were recorded. At 12.1ppm, gill showed hyperplastic cells, necrosis, hyperplasia, clubbing of the secondary lamella, hypertrophy, erosion and degeneration of secondary lamellae, whereas loss of the shape of the hepatocytes, vacuolization, cell membrane lyses, cytoplasmolysis and hypertrophy in liver were seen. The damage showed concentration dependent response.